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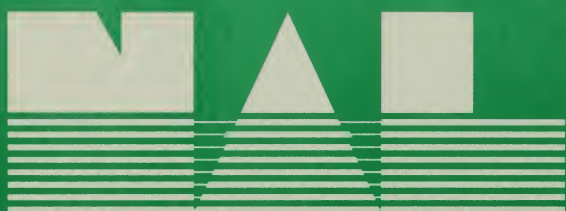
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**PROCEEDINGS OF THE
1990 SUGAR PROCESSING
RESEARCH CONFERENCE**

**MAY 29-JUNE 1, 1990
SAN FRANCISCO, CALIFORNIA**



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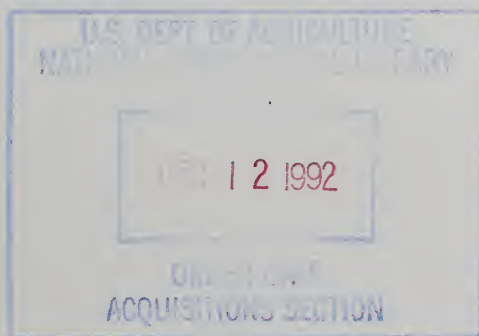
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**Sponsored by
Sugar Processing Research, Inc.**

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PREFACE

The 1990 Sugar Processing Research Conference is one of a series of Conferences held in alternate years to provide a forum for exchange of information among technical leaders of the sugar industry and to report on new and noteworthy developments. The Conference was sponsored by Sugar Processing Research, Inc. (SPRI). Contributions from the Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture are gratefully acknowledged.

The 1990 Conference celebrated the Fiftieth Anniversary of the founding of SPRI's predecessor, the Bone Char Research Project. Remarks by Dr. Victor R. Dietz, the scientist who began the Bone Char Research Project, are included as a foreword for this Proceedings.

The program for this conference was arranged by Margaret A. Clarke. The Conference Coordinator was Shirley T. Saucier. These proceedings were edited by Margaret A. Clarke, with editorial assistant Beryl Ann Borel.

These Proceedings are published by Sugar Processing Research, Inc. The series Proceedings of the Sugar Processing Research Conference, of which this is the fifth issue, continues the Proceedings of the Technical Sessions on Cane Sugar Refining Research, which was published every other year from 1964 to 1980. For individual copies of this volume as well as back issues of the former series as long as the supply lasts, write to Sugar Processing Research, Inc., P.O. Box 19687, New Orleans, LA 70179.

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FOREWORD

THE FIRST DECADE OF THE BCRP

Victor R. Deitz

The records indicate that BRCP (Bone Char Research Project) was conceived in 1937 by John W. Lowe. The project operated on a "gentlemen's agreement" until incorporated under the laws of the Commonwealth of Massachusetts on December 20, 1948. The official name was United States Cane Sugar Refiners' Bone Char Research Project, Inc. The account which follows deals with the period 1937-1948: the early beginnings, initial activities, and the status at the time of incorporation.

John William Lowe was born in New Haven in 1888. He graduated in chemistry at Yale 1909 and served as Chief Chemist for the Warner Sugar Refining Co., New York from 1910 to 1918. His boss was L.J. Seidensticker who inoculated Mr. Lowe with an insatiable intellectual curiosity regarding the bone char process in sugar refining. In 1918 he became Assistant Superintendent of the California and Hawaiian Sugar Refinery in San Francisco, but returned east in the same year to the newly formed Revere Sugar Refinery. He advanced successfully to become Assistant Superintendent, Superintendent, Refinery Manager, and Vice President.

John Lowe was first to emphasize the danger of the industry's reliance on a unit process that was being operated in semi-ignorance. He wrote a letter dated May 20, 1937 to Dr. Lyman J. Briggs, Director of the National Bureau of Standards (NBS).

"... For a long period of years I have advocated that our industry undertake some research work in the realm of pure science, particularly with reference to the physical and chemical characteristics of animal bone black. This is a subject which has been shrouded in mystery and about which, from the standpoint of pure science, we have learned little or nothing in the last fifty years. My hope is that some day our industry will undertake this work through some medium such as the Massachusetts Institute of Technology, the Mellon Institute, or the Bureau of Standards..."

Mr. Lowe maintained persistent contact with Frederick J. Bates of the National Bureau of Standards. Mr. Bates was quite sympathetic with the needs of the sugar industry having been in close association with the industry since the establishment of the Polarimetry Section at NBS. Mr. Lowe obtained the promise of the Bureau's support working through its Research Associated plan of cooperation of Government with Industry. In 1937 there were 26 such projects at NBS employing a total of 45 scientists, the

largest being that sponsored by the American Petroleum Institute under F.D. Rossini, well-known in chemical thermodynamics.

Mr. Lowe undertook a one-man educational program in 1937 to convince his colleagues of the refining industry for the need of some centralized research group to obtain the basic information. His pitch for the establishment of the research was as follows:

1. Universal ignorance on the part of the sugar refining industry of the color-removal step, despite long usage of bone char in refining (since 1828).
2. Persistent occurrence of occasional very good and occasional very bad colorization at all refineries without apparent reason.
3. The financial investment in char was sufficiently large to demand some technical assurance that it was being used at optimum efficiency.
4. The scientific information was needed by all refineries, but none had the time, the scientific and skilled personnel, nor the laboratory facilities to make a start.

Mr. Lowe's perseverance was climaxed when his firm put up the first contribution of \$1000, which was matched immediately by three other charter members from among the refiners: Ben Sprague (Savannah), William Hoodless (Pennsylvania), and Lewis Placet (McCahn). With \$4000 in the bag, Mr. Lowe requested Mr. Bates (June 1938) to recruit a scientist to undertake the laboratory work. He also brought the project to the attention of the bone char manufacturers and suggested that they become sponsors. Mr. Lowe reported (6 June 1938) that three refiners had refused to participate and three were on the fence. However, when the first meeting of the research committee was held in May 1939, there were 11 subscribers: 8 refiners and 3 bone char manufacturers. It is interesting to note that today, 50 years later, only two of these sponsors have survived the intervening business hardships and these two sponsors are still listed in today's program notes.

The Research Committee appointed J.M. Brown as chairman, a very fortunate appointment. Jim was Chief Chemist of the Revere Sugar Refinery and with Joe Bemis had published several papers on several aspects of the Bone Char Process. They assembled a list of research problems pertinent to the refining process and submitted these to F.J. Bates as suggested topics for the contemplated work at NBS. This list is given below, and when examined 50 years later, the writer has a guilty feeling that some unanswered problems still exist. Fortunately, the subsequent researchers of Frank Carpenter and Margaret Clarke have brought all problems into better focus.

A. RESEARCH PROBLEMS SUGGESTED IN 1938

A detailed literature survey on bone char and solid adsorbents were deemed necessary before the laboratory work could be started. This was published as the "Bibliography of Solid Adsorbents."

1. Nature of active carbon and inactive carbon, specifically as to sorptive capacity.
 - a. Area of availability carbon surface
 - b. Cleanliness of surface
 - c. Distribution in the particle
 - d. Arrangement of the molecular structure
2. Structure of bone char - concerning macropores
 - a. Order of magnitude of diameter
 - b. Specific macropore area, i.e., total area of carbon per unit volume or weight
 - c. Are macropores completely interconnected?
3. Structure of bone char relative to micropores
 - a. Cross sectional area
 - b. Specific micropore area
 - c. Are micropores completely interconnected?
4. Nature of exchange reactions taking place between the char and colloidal particles in the liquor such as coloring matter. Optimum pH at which was water is to be held. Relation between pH of char (water extract) and its selective and varying adsorption. Amphoteric electrolytes. Nature of reactions.
5. Order of ash adsorption preference for salts commonly found in raw sugar.
6. Determination of standard and definite methods of analysis and evaluation of char, including standard methods to evaluate shrinkage and discard values.
7. Study of porosity and its effect on activity and revivification. True density-microscopic technique.
8. Relation between particle size and activity of char, i.e., study of granular size of bone char used in sugar refineries as regards decolorizing and ash removal results; also relationship between various grist sizes and fluid flow of sugar liquors at various temperatures and densities.

B. PROBLEMS OF PRACTICAL VALUE IN 1938

1. Critical temperature of char revivification. Temperature of over-burning. Rate of reaction of over-burning. Conditions of over-burning. (Reduction of sulphates).
2. Critical temperature of char revivification in relation to contact time.
3. How does degree of exhaustion due to prolonged filtration of dark colored materials affect the subsequent revivification of bone-char?
4. Relation between moisture content of char entering a retort and the degree of revivification.
5. Chemical or other means of reactivation to increase efficiency.
6. Effect of impurities on reactivation and subsequent selectivity. Iron compounds, sulphates, etc., and their effect on color and ash removal.
7. The manner of selective adsorptivity of char for various color compounds in sugar products.
8. Adsorption of bacteria or spores by char.
9. Relative efficiency of bone-black on defecated liquors, such as liquors clarified by the Williamson process, or vacuum-continuous filters using P_2O_5 , liquors prepared by mechanical filtration only, and liquors with addition of Perone.
10. Investigation of the following theories
 - a. Perone reaction
 - b. CO_2 impregnation
 - c. Heat rise on saturation of char with liquor.
11. Optimum calcium carbonate content of char.

The first open meeting of the Subscribers of the Project and the Research Committee took place on December 6, 1939 at the Hotel Lexington, New York. The attendance was 14. The many topics raised during an active question-answer period served to direct future studies of the project.

It was not until June 1942 that a second meeting could be held because of the many war efforts of the sugar industry. Sugar always becomes a scarce item in war times and a surplus in peace times!

The first laboratory measurements were concerned with the surface areas of new and service bone chars. We were able to place as sequence of samples, new to discard materials, in direct proportion to the BET surface area. However, when compared with the results for a batch-type decolorization test of the same samples, it was found that sometimes the decolorization was proportional to the BET areas and sometimes it was not. Obviously, the decolorization process was far more complicated than could be accounted for by the extent of surface.

With the ending of World War II, it was decided to examine in detail the commercial bone char process as conducted in three industrial units: (1) Pennsylvania Sugar Refinery, Philadelphia, PA, (2) Refined Syrup and Sugars, Yonkers, NY, and (3) Revere Sugar Refinery, Charleston, MA. Three types of kilns were available at these locations to revivify bone char: (1) Kilns with Stationary Retorts, (2) Herreshoff Multiple Hearth, and (3) Rotary Kiln used as a Decarbonizer. The observations made in 1945 were published in book form (301 pp) in 1947 under the title "Survey of Bone Char Revivification and Filtration."

A number of important problems were raised. One general conclusion was that there were probably several procedures which are capable of bringing about a successful revivification of bone char. A kiln, designed after making compromise to many conflicting factors, need not necessarily be constructed in a unique manner. With adequate technical control imposed by a competent scientific staff it is possible that improved procedures could be placed in operation based on the use of a Herreshoff furnace, a rotary kiln, a kiln with stationary retorts, or some combination of the principles involved in these three methods. All of these could prove to be equally satisfactory from a technological and practical point of view.

The final venture of the BCRP in the period under discussion was the comprehensive study of char house operations based on visits to 24 refineries and correspondence with three additional. These were made with S.E. Cotter and the writer. The object was to present a general analysis of char house operations that existed in 1945. The counsel and advice of Jim Brown was most valuable in this task; the observations collected in the study are given below:

1. Melt
2. Char stock

3. Total number char filters and description (dimensions, char capacity, liquor capacity, filter tops, false bottoms, blankets and exit valves)
4. Systems of char and how divided
5. Method of settling filters and time of filling with each system of char
6. Time schedule and grades of liquor-on with each system of char and grades of liquor-off each system of char
7. Liquor flow rates and method of control, pressure on filter head floor
8. Brix, purity, temperature and pH of liquors-on and liquors-off each system of char
9. Method, rate and time sweetening-off from water-on to sweet water-out, conditions of final sweet water
10. Rate and time of washing to sewer, conditions of final sewer water to conclude washing, temperature of water, water treatment, wash water outlet to sewer
11. Driers: number, make, now divided among systems of char, moisture in and out of drier
12. Kilns: number, make, how distributed in systems of char, arch, number retorts in rows, number rows, type and dimensions of retorts
13. Type of fuel and manner of temperature control, temperature of kiln air and location of thermocouples
14. Method of decarbonization
15. Method of cooling char (cooler pipes and supplemental cooling) and temperature of char entering filter, utilization of hot air surrounding cooler pipes
16. Type of draw mechanism, rate of draw and temperature of exit char
17. Method and amount of dust removal, screen analysis of dust and of service chars, method of discard grains, if any (S.S.&S.)
18. Conveying and elevating equipment
19. Density of char from each char system and the chemical analyses of each

20. New char injection rate
21. Percent char burned

The total stock of service bone char that existed in 1945 was 124,000,000 pounds with which to refine a potential daily melting capacity of 74,000,000 pounds. The progress towards modernization of the char house had been relatively slow. The factors which had led some increase in char efficiency over the previous 25 years were:

1. Increase in purity of the raw sugars
2. More efficient affining due to higher-speed centrifugals
3. Better clarification before char filtrations
4. Better control of flow rates
5. Control in kilning parameters
6. Decarbonization and cooling of char.

It should be of interest to current operations after the intervening 40 years to compare the present status where possible with that reported in 1945. The primary data are available to those so interested.

In 1948 the available funds were increased and additional staff members were recruited. The total funds expended from 1939 to 1948 was \$80,000. Dr. Frank Carpenter came aboard on October 11, 1948 and Neil Pennington on September 1, 1947. There were many others to whom the writer will always be grateful for their contributions. The Technical Sessions were started in 1949 and a good time was had by all.

GROWTH AND MORPHOLOGY OF SUCROSE CRYSTAL

Giorgio Mantovani

University of Ferrara

As is known, the sucrose crystal shows 15 simple forms which have been determined with certainty, some of them being more important and common compared with the others. The study of these forms was carried out through the preparation of monocrystals of different sizes grown both in pure and impure mediums.

However, for a correct interpretation of the habit modification experimentally observed, we judged it indispensable to study, in strict cooperation with colleagues of the Department of Earth Sciences of the University of Turin, the theoretical morphology by applying the theory of Hartman and Perdok, (Aquilano et. al, 1983).

Through the identification of the periodic bond chains, (PBC) (Figure 1), and by assuming for the first approximation the hydrogen bonds were unique strong interactions between first neighbours and not taking into account the entropic contents, it was possible to point out the F or S characters of the different faces present in the crystal, (Figure 2). We can observe that some forms, which have an S character and which consequently might not appear in the crystal growth morphology, are actually always present. In particular the form d- {101} is always present in the crystals grown from pure solution and sometimes shows a considerable width.

In reality, although according to the analysis of H. & P. this form has a pure S character, (Figure 3), (Aquilano et. al, 1986) by successively considering the molecular interactions, (hydrogen bonds and Van der Waals bonds between second and third neighbours), it acquires a weak F character, (Aquilano et. al, 1987). Moreover, if we take into consideration also the interactions of the molecules of water, which is the solvent of the growth solution, we can show, (Figure 4) (Vaccari et. al, 1986), the theoretical existence on the form of three new PBC's which give it a strong F character so justifying its presence in the growth morphology. The relative width of this form as a function of the others, even in pure solution, is certainly to be correlated to the growth conditions and in particular to the temperature and supersaturation conditions (Figure 5). Another strict correlation occurs between the width of this form and the presence in the solution of particular impurities of inorganic type, that is for instance KCl (Figure 6). A possible interpretation of this phenomenon could be in this case the possibility of duo-dimensional epitaxy phenomenon of KCl on the face itself, whose growth is consequently blocked (Figure 7), (Aquilano et. al, 1984, Aquilano et. al, 1987).

The sucrose crystal polarity is well emphasized by the specific effects of raffinose which, if present in the solution, completely blocks the p' faces growth without interfering with the p faces (Figure 8 a,b), (Vaccari et. al, 1986). This effect becomes emphasized by observing figures 9 and 10 which show the interferometric holograms in pure solution and in the presence of raffinose, respectively. These holograms were experimentally obtained in cooperation with colleagues of the Mineralogy Institute of the University of Genova. It is worth pointing out that the effect of raffinose is not confined to poisoning the p' faces but also interferes to a certain extent with some of the faces of the [hOL] zone so that the crystal becomes elongated along the b axis as shown in figure 11.

Raffinose, by affecting the growth spirals on the "a" face, causes the characteristic phenomenon known as tapering of the sucrose crystal as shown in figure 12, (Bedarida et. al, 1988). Particularly interesting is the effect of raffinose on the sucrose twins whose morphology changes with time moving from the first type twins, (the right poles outwards), gradually to the second type ones (twins grown together along the (100) face), and to the third type twins (the left poles pointing outwards) (Figures 13 and 14), (Mantovani et. al, 1983, Vaccari et. al, 1986).

From the above comments it is clear that the crystal morphology, which is strictly related to the growth kinetics of the different faces, is influenced both by the growth conditions and the presence of particular impurities. During the industrial production of sucrose, the amount and the quality of the impurities can notably vary as a function of the different quality of the raw matter and, in particular, if we are processing beet or sugar cane. In the first case the crystals have a morphology which is fairly close to the one we can observe in pure solution whereas in the case of sugar cane the crystals are often elongated along the c -axis (Figure 15). As well as an elongation along the c -axis, cane sucrose crystals originating from low boiling present also a peculiar morphology that is the appearance of some rare faces on the right pole and the disappearance of some other ones on the left pole (Figure 16) (Vaccari et. al, 1989a). This particular D-shape has to be related to the high amount of invert which can be found in the cane processing (Figure 17), (Vaccari et. al, 1989b)

Taking into account that, as previously shown (Mantovani et al., 1985a,b, Mantovani et al., 1986a,b), the faces growth kinetics is strictly related to the possibility of inclusion of coloured mother liquor, the different crystals' morphology affects the different location of colour inside the crystals. In the particular case of beet sugar production, whose p' faces grow more rapidly, the inclusion of mother liquor, and consequently of colour, interests in particular these faces (Figure 18). In the

case of cane sugar, whose rapidly growing faces are the ones which grow along the c-axis, the colour location occurs along the direction of this axis, (Figure 19).

These comments about the colour of sucrose crystals are an example of how important is, also from a technological point of view, the study of crystal morphology and its modifications as well as the crystallization parameters and the presence of impurities. Concerning this last point, on the grounds of laboratory experiments, we planned a cooling crystallization process, (Mantovani et al., 1988) in which crystallization is realized through a temperature decrease instead of evaporation as in the traditional boiling pans, (Figure 20). The results we obtained pointed out that it is possible to directly obtain crystallized sugar of commercial quality starting from thick juice with an energy consumption which is in favour of cooling crystallization. On the grounds of the results obtained through these preliminary tests we set up a pilot plant able to produce one ton of sugar per day, (Figure 21), (Vaccari et al., 1988). This plant was in operation during a whole sugar campaign, and the results confirmed what was seen in the laboratory tests, that it is possible to obtain white sugar of commercial quality directly from thick juice even if this latter has unfavourable characteristics as far as colour is concerned.

Taking into account the remarkable advantages achievable by cooling crystallization we have carried out preliminary laboratory experiments with the aim of obtaining white sugar directly from raw juice. The results of such experiments which will be discussed in Eastbourne at the next Sugar Tech 1990 (Vaccari et al. 1990), seem to give the possibility of obtaining commercial sugar at low cost price by completely eliminating the onerous and complex juice purification steps which cause too pollution problems (Figure 22).

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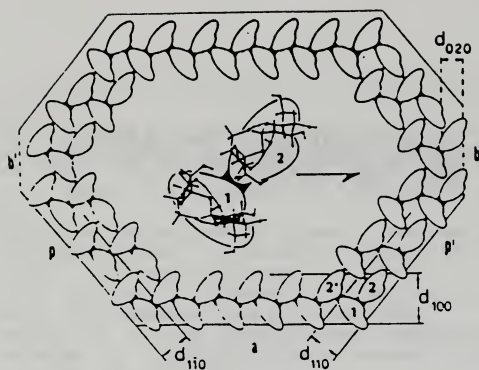


Figure 1.-- [001] projection of a sucrose crystal: the PBC $[001]_A$ built up by the chain couple 1-2 is drawn at the centre of the figure.

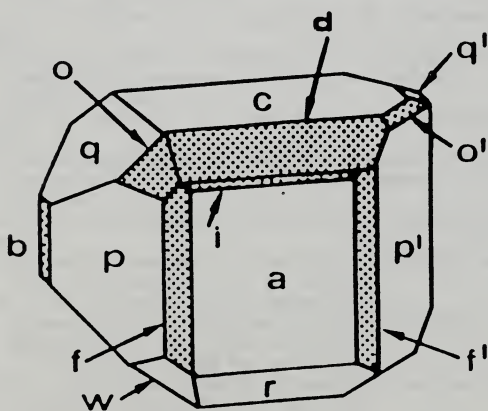


Figure 2.--Theoretical growth morphology of a sucrose crystal. Dashed faces are those showing S character.

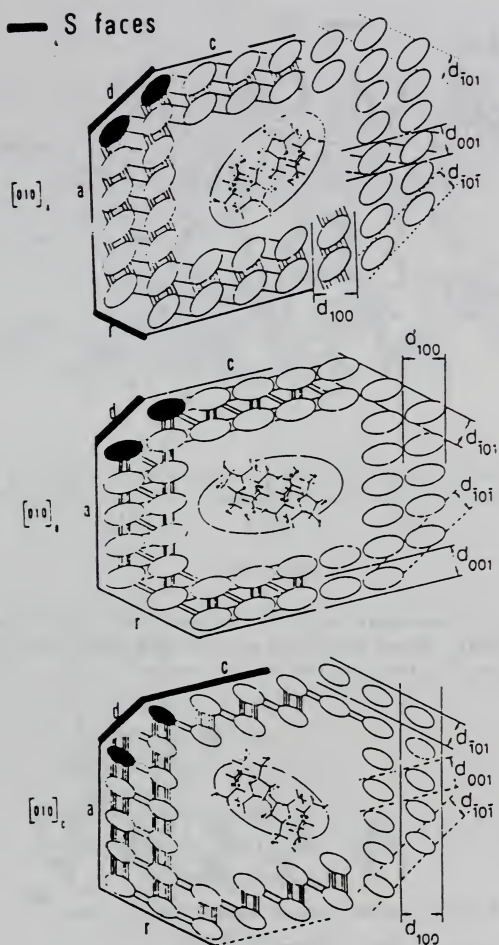


Figure 3.--Projection of the sucrose crystal structure along the $[010]$ direction according to the PBC's $[010]$ A,B,C. The PBC's related to the d face which are never directly bound are indicated.

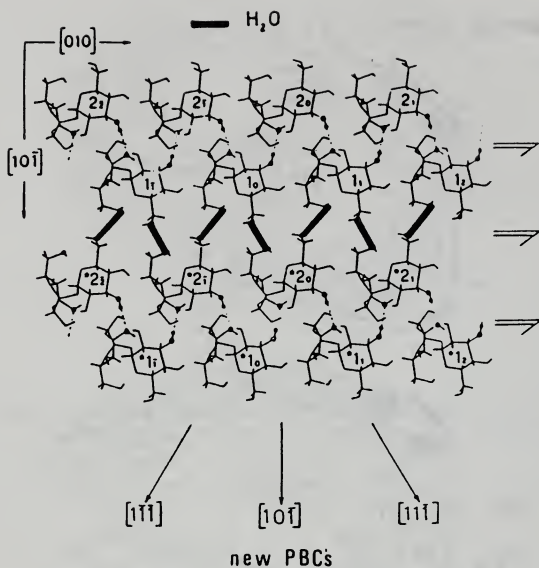


Figure 4.--Outermost layer of the face (101) showing the profile (010). Water molecules settled down among the [010] PBC's, thus creating new PBC's.

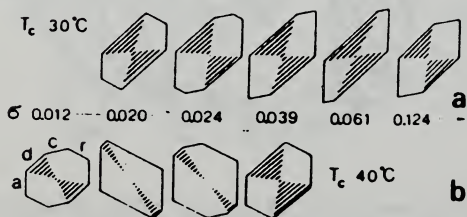


Figure 5.--Steady state growth form and growth sectors of the d-form, in the {h0L} zone at 30°C (a) and 40°C (b).

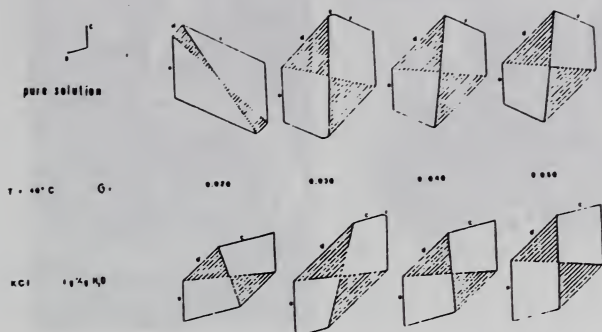


Figure 6.--Habit modification, in the $\{h0l\}$ zone, induced by KCl (4 g/g water) in comparison with pure solutions.

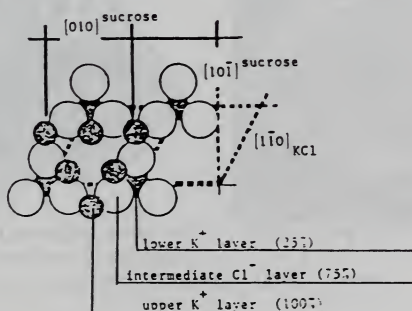
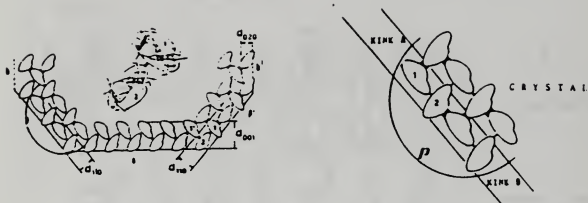


Figure 7.--Epitaxial coincidence between $[010]$ plane of sucrose and $[111]$ plane of KCl.



A



B

Figure 8.--[001] projection. A. The bonds involved in the p slice are: $2(\epsilon, \gamma), \beta, \alpha, \delta$. B. The bonds involved in the slice p' of monomolecular thickness are: $2(\epsilon, \gamma), \alpha, \delta, \beta$.

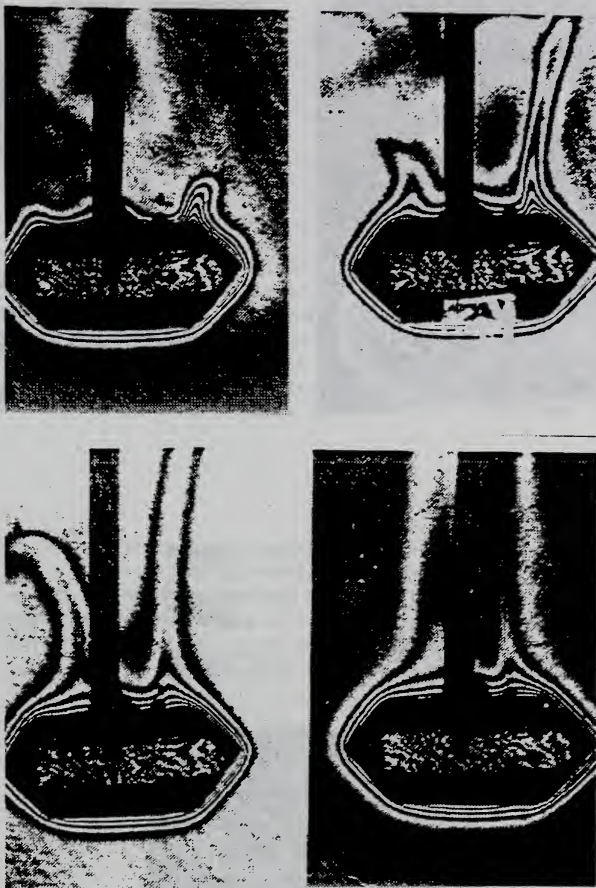


Figure 9.--Four interferometric holographic images of a sucrose crystal growing from pure solution ($T = 20.5^{\circ}\text{C}$, $\sigma = 0.1$); the time interval between every pair of consecutive images is 30 minutes.



Figure 10.--Two interferometric images of a sucrose crystal growing from a solution with raffinose (4 g/g water), $T = 19^{\circ}\text{C}$, $\sigma = 0.13$ obtained at a time interval of 18 hours. By the interference fringes it can be easily seen that the sphenoid $\{1\bar{1}0\}$ is growing, whereas the growth of the sphenoid $\{110\}$ and of the pinacoid $\{100\}$ is stopped.



Figure 11.--Characteristic habit of a sucrose crystal grown in the presence of raffinose.

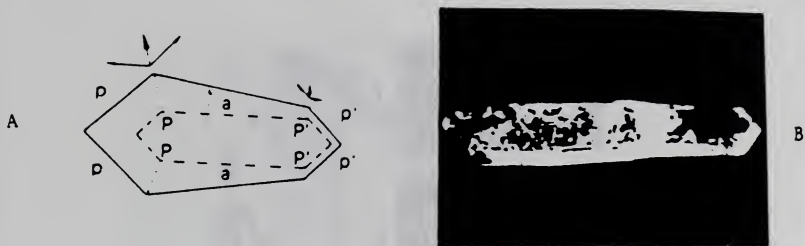


Figure 12.-- A. Scheme of the crystal view along the $[001]$ direction. Dotted lines represent the theoretical profile of the crystal growing in pure aqueous solution; full lines represent the experimental profile of the crystal growing in the presence of raffinose. B. Sucrose crystal grown in the presence of raffinose viewed along c -axis.

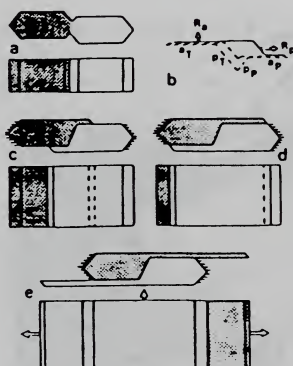


Figure 13.--Schematic drawing of sucrose twin grown in the presence of raffinose: (a) initial seed shape; (b) filling up of the re-entry angle ($R_p/R_a = \infty$); (c), (d) successive stages of twin penetration. The habit represented in (d) looks like that of a 2nd type twin; (e) final stage of growth: p forms overhang p' forms. Final habit looks like that of a 3rd type twin, p forms pointing outwards. (P = parent; T = twin).

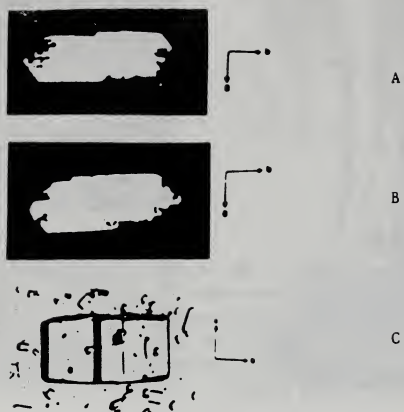


Figure 14.--Sucrose crystal shown: A: in figure 13a; B: in figure 13c; C: in figure 13e.

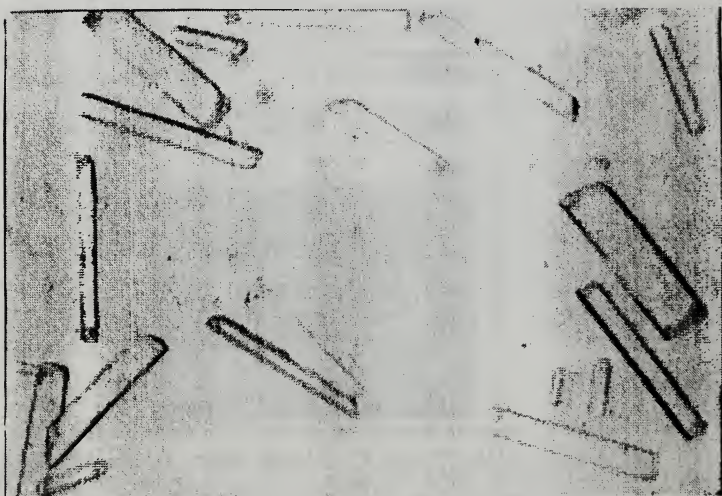


Figure 15.--C massecuite sucrose crystals from cane sugar refinery.

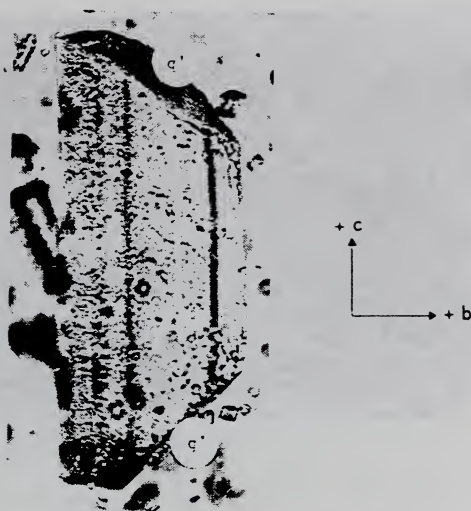


Figure 16.--Particular D-shape shown by C-massequite sucrose crystals from cane sugar factory. There are no o, q and w faces on the left pole whereas the q' faces on the right pole are quite evident.

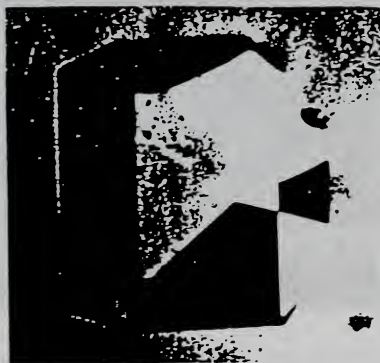
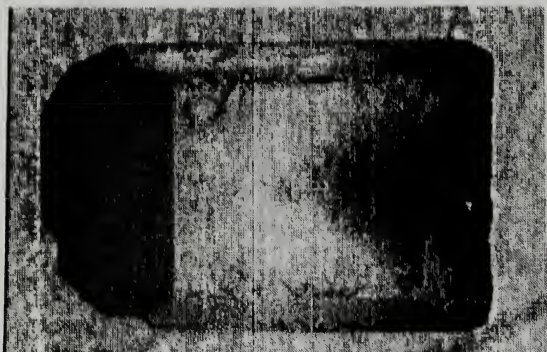


Figure 17.--Sucrose crystal grown from a sugar solution in the presence of glucose and fructose both of them at the concentration of 100 grams per 100 grams of water.



A



B

Figure 18.--Inclusion of colouring matter on p' faces from the beginning of nucleous formation. A: single crystal; B: twin.

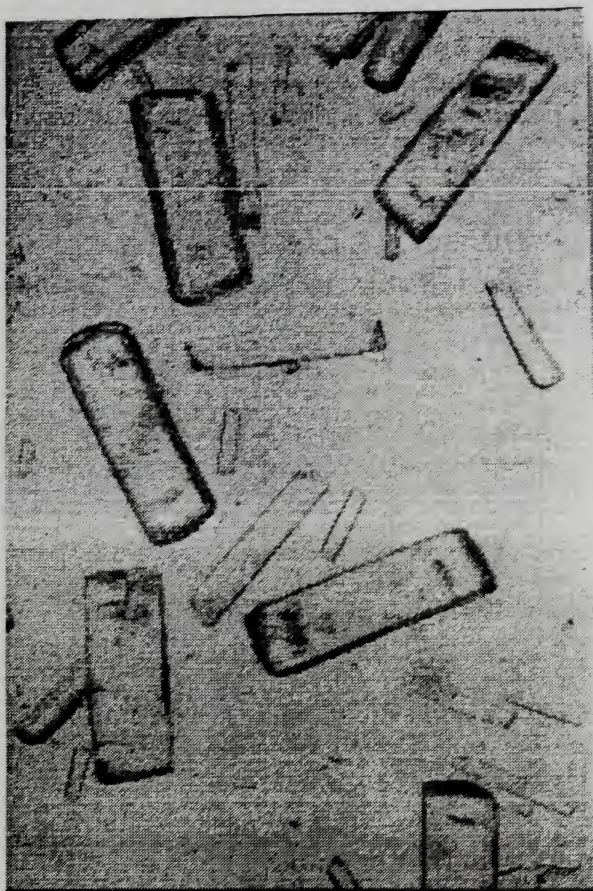


Figure 19.--Cane sucrose crystals: colouring matter inclusion along the c-axis.

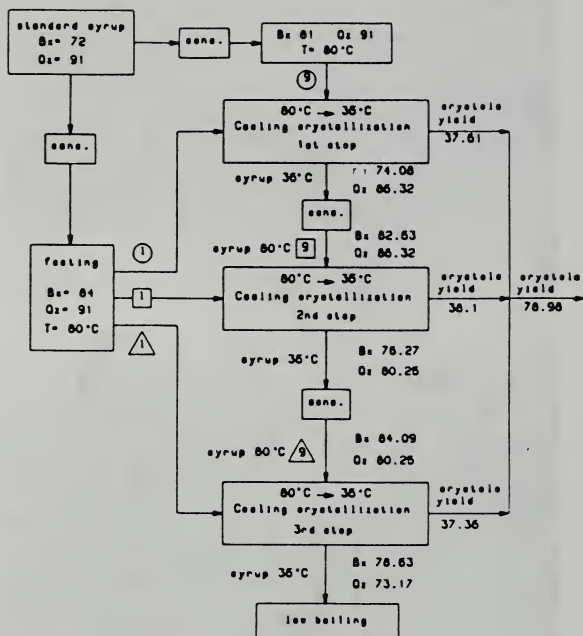


Figure 20.--Basic scheme of a cooling-crystallization process in 3 stages.

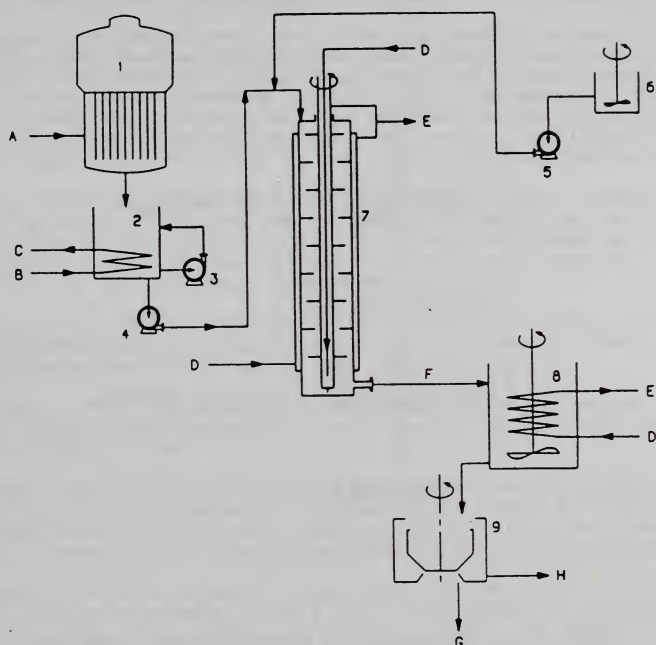


Figure 21.--General scheme of the CCC pilot plant. 1. Boiling pan. 2. Tank for collecting the standard syrup. 3. Recirculation pump. 4. Pump at variable number of revolutions. 5. Peristaltic pump. 6. Tank for storing the slurry of seed in isobutyl alcohol. 7. Crystallizer. 8. Tank for collecting magma. 9. Centrifuge. A. Standard syrup. B, C, D, E, Cooling water. F. Magma leaving the crystallizer at about 50°C. G. Commercial white sugar. H. Syrup leaving the centrifuge.

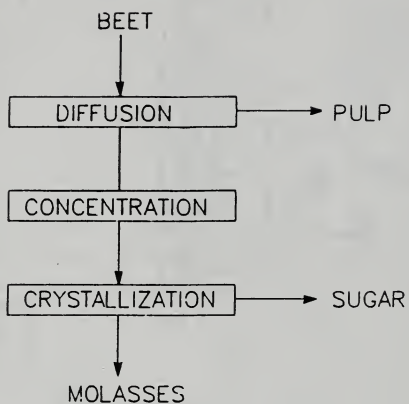


Figure 22.--Flow diagram of a hypothetical beet sugar factory adopting the raw juice direct crystallization.

DISCUSSION

Question: What can your studies on morphology tell us about the sort of seed crystal we use and its effect on the crystal?

Mantovani: Our experience concerning utilization of seeding showed us that when we employ seed traditionally prepared through milling we can observe, in the first steps of crystallization, inclusion of coloured mother liquor. This phenomenon is due to the fact that the irregular crystal surface, which follows the milling step, becoming smooth thanks to growing, includes colored mother liquor droplets owing to the higher crystallization rate.

The best condition would be spontaneous nucleation. However, colouring matter inclusion can be limited by adopting conditions allowing low growth rate in the first growth step such as, for instance, by using cooling crystallization. This technique has been adopted, for instance, by B.M.A.

Question: On cooling crystallization in 3 steps: When you begin with a slurry, and you drop from 80° to 35°C, what is the crystal size distribution at the end of the cooling stage?

Mantovani: The crystal size at the beginning is the normal crystal size of seed because it is a slurry, about 0.05. This is grown to about 0.4 mm.

Question: That is a very fine grain; what we need is grain bigger than 0.5 millimeters to handle in storage. I think that is a very important question. In my opinion, it is not possible to make a good crystal size distribution by only cooling steps.

Mantovani: We have prepared fine grain because in Italy the market asks for crystals of this size. Of course we could obtain bigger crystals by suitably checking the amount of seed. As far as the crystal size distribution obtained by cooling is concerned, no difficulties would be met if the cooling rate is so adjusted that secondary nucleation phenomena are avoided. We have observed this picture in our laboratory experiments.

Question: We talked about melassigenic factors of various non-sugars, e.g. potassium chloride, with a coefficient of 5 or 6. The phenomena that slows down crystal growth is that the same phenomena that maintains sucrose in a soluble state by preventing an effective nucleation of the sucrose? Are these the same effects of solubility: an increased solubility in potassium chloride, versus a slowing down or changing of the rate of the crystal face growth?

Mantovani: Potassium chloride slows down crystal growth because, by increasing sucrose solubility, decreases the supersaturation coefficient. As far as sucrose crystal habit modification, we have studied the influence of potassium chloride only for explaining from a theoretical point of view the width of the d-form. This is the only habit modification we can observe in the presence of potassium chloride which cannot be considered a real habit modifier such as, for instance, raffinose, some oligosaccharides or polysaccharides.

Question: I think you said that, about the inclusion of colorants in a crystal face that is growing very rapidly, there is a correlation between the inclusion of colorants and rapid growth of the crystal face.

Mantovani: Yes.

Question: Are these colorants incorporated on a molecular basis or simply as a pool of liquid, as Powers indicated was included in the crystal?

Mantovani: Normally as droplets of the solution, when you do this at a very high rate of crystallization. Inclusion does not depend on the coloring matter but on the rate of crystallization of the various faces.

Question: Can you also expect in such crystals a higher degree of internal moisture and other non-sugars?

Mantovani: Yes. Very often high color and high ash contents occur together for example. Water is normally included inside sucrose crystals.

STRUCTURE AND PROPERTIES OF SUGARBEET PECTINS

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INTRODUCTION

Apple pomace and citrus peels are until now the only sources of commercial pectins. Sugar-beet pulp has been investigated as an alternative source (Kertesz, 1951); it is the residue left after sugar extraction from sugar-beet and in its dry form, it is available all year round at relatively low prices. Some 25% of its dry weight consists of galacturonic acids and it is therefore a potential source of pectins. However, attempts in the past to commercialize sugar-beet pectins have failed, because they have poor gelling properties, compared with citrus and apple pectin. The poor gelling properties were ascribed to the presence on the pectin molecules of acetyléster groups (Pippen et al., 1950) which were characteristic for these pectins.

Our recent studies on sugar-beet pectins have revealed some new structural features, especially the presence of feruloyl groups and a new way of gelatin specific for feruloylated pectins was proposed (Rombouts et al., 1983) as crosslinking reactions through oxidation of these phenolic groups can be carried out. It has been known for some time that ferulic acid, cinnamic acid, and tyrosine substituents bound to biopolymers may be involved in the formation of crosslinks in the presence of certain oxidants. For instance, wheat flour pentosans contain feruloyléster groups, and the formation of diferulic acid crosslinks by addition of hydrogen peroxide/peroxidase results in gel formation (Geissmann and Neukom, 1973). Similar reactions were used for photocross-linking of polymers such as cellulose and polyvinyl alcohol on which cinnamic acid residues were bound (Delzenne, 1969). Following up on these observations, we have succeeded in cross-linking sugar-beet pectins by using some specific agents. Therefore, the usefulness of the sugar-beet pectins was increased and some new applications may be found as it is possible to increase the viscosity of their solution or to produce gels.

The aim of this chapter is to recapitulate the work we have done on these pectins, their extraction, their chemical structure, the location of feruloyl groups and their oxidative crosslinking, and on the properties of the modified pectins.

EXTRACTION AND CHEMICAL STRUCTURE

Extraction and chemical composition. Very low quantities of pectins are solubilised by water (2.2%) and by calcium-chelating

agents (0.5%) from an alcohol-insoluble residue (AIR) of sugar beet pulp (Rombouts and Thibault, 1986a) and it is likely that the corresponding fractions have been eliminated during the leaching process (Le Quéré et al., 1981; Thibault, 1988). The bulk of the pectin is solubilized by hot (85°C) dilute (0.05N) acid (HCl) (~20% of the AIR) but a significant amount (~11% of the AIR) is further extracted with cold (4°C) dilute (0.05N) alkali (NaOH). These two steps extract 95% of the galacturonic acids initially present in the AIR. Their characteristics are shown in Table 1.

TABLE 1.-- Characteristics of the pectins

	pectins			
	water soluble	oxalate soluble	acid soluble	alkali soluble
yield (% of AIR)	2.2	0.5	19.9	11.1
Galacturonic Acid	54.4	77.9	65.1	54.9
Rhamnose	0.9	0.9	2.2	3.2
Arabinose	8.4	1.8	10.0	12.5
Xylose	0.1	0.2	0.2	0.2
Mannose	0.2	0.1	0.1	0.0
Galactose	6.5	2.4	5.9	8.1
Glucose	0.4	0.2	0.4	0.3
Feruloyl groups	0.1	0.0	0.5	0.6
Degree of methylation	75.5	59.7	61.8	7.5
Degree of acetylation	31.3	15.5	34.5	4.0
Viscosity molecular weight	47,700	15,400	42,800	36,400

The beet pectins have fairly low molecular weights in the range of 15,000-48,000 (Michel et al., 1985; Phatak et al., 1988; Dea and Madden, 1986) and relatively high content (6-24%) in neutral sugars when compared to apple and citrus pectins. Arabinose, galactose and rhamnose are the main sugars in all the fractions. The molecular weight of beet pectins could partly explain their low gelling power. However, the high degrees of acetylation (up to 35%), found especially in the acid-soluble fraction, seems to be the main reason in preventing the close association of polymers required for the gelation (Rombouts and Thibault, 1986a). Hydroxyproline-rich proteins (1-2%) are present in the pectins, even after their purification by ion-exchange chromatography (Guillon and Thibault, 1988). In addition, feruloyl groups are found in low quantities (<1%); these phenolic acids are ester-linked to the pectins (Rombouts and Thibault, 1986a) and are not present on pectins from apple, citrus, cherry, and potato (Rombouts et al., 1983), with the exception of spinach (Fry, 1983).

which belongs to the same botanical family as sugar beet. The presence of feruloyl groups in the sugar-beet pectins was shown (i) by spectroscopy (Rombouts and Thibault, 1986a) (Figure 1) which revealed a bathochromic shift of the double absorption peak at 300 and 325 nm at pH 4.8 towards a single peak at 375 nm at pH 10, this shift being typical of esters of cinnamic acid-type phenols, and (ii) by high-performance thin-layer chromatography (Rombouts and Thibault, 1986a) and high-pressure liquid-chromatography (Guillon and Thibault, 1988) after hydrolysis and extraction of the liberated (ester-linked) phenolic acids which have shown that ferulic acid is the only phenolic acid ester-linked to the pectins.

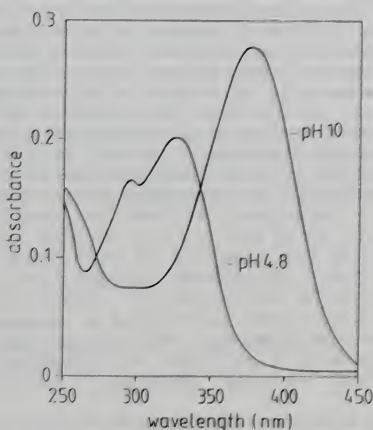


Figure 1. Absorption spectra of sugar-beet pectins

Fine structure. Chemical analysis showed that beet pectins are rich in galacturonic acids, arabinose and galactose, with a high content of acetic acid and a low but significant content of ferulic acid. In order to have a better insight into their chemical structure, highly purified enzymes have been used (Rombouts and Thibault, 1986b). The results obtained with depolymerizing enzymes (endo-polygalacturonase and pectate-lyase) after chemical deesterification confirmed what is known already for other pectins from apples (De Vries et al., 1982), citrus (Axelos et al., 1989) and cherry fruits (Thibault, 1983); the neutral sugar side-chains attached to the rhamnogalacturonic acid

neutral sugar side-chains attached to the rhamnogalacturonic acid backbone occur in blocks, the so-called "hairy" fragments, leaving large parts of the main chain unsubstituted ("smooth" regions). The location of the acetyler groups has been studied by analysing the products of pectin hydrolysis after sequential treatment with pectin-methylesterase and endo-polygalacturonase and with exoarabinanase and endo-galactanase. The results show that 80-90% of the acetyl groups are linked to the "smooth" regions through C₂ and/or C₃ of the galacturonic acid units, and are fairly regularly distributed along the chain (Rombouts and Thibault, 1986c). In contrast, it was shown that the feruloyl ester groups were mostly recovered in the "hairy" fragments, while only part of the hydroxyproline-rich material was found in these fragments (Rombouts and Thibault, 1986b; Guillon and Thibault, 1988, 1989).

The fine structure of the side-chains and the location of the feruloyl groups in the "hairy" fragments were studied by chemical and enzymic methods (Guillon and Thibault, 1988, 1989; Guillon et al., 1989). Methylation analysis showed that acid-soluble and alkali-soluble pectins as well as their "hairy" fragments have closely related chemical structures. The backbone of the "hairy" fragments consists of α -1,4-linked galacturonic acid residues with 1,2-linked L-rhamnosyl residues. The C₄ of the rhamnosyl residues is the main point of attachment of the side-chains. Arabinose residues, mainly in the furanose form, are terminal, 1,5- and 1,3,5-linked. Galactose residues are mostly 1,4-linked with few branched points on C₃ but 1,3-, 1,6- and 1,3,6-linkages are also found indicating the presence of both types I and II (arabino)-galactans. These structural features are consistent with the results obtained by Keenan et al. (1985) from ¹³C-n.m.r. which have shown that sugar-beet pectin has a backbone of a 1,4-linked α -D-galacturonan containing a small proportion of rhamnose with side-chains composed of 1,5-linked α -L-arabinofuranosyl residues and β -galactosyl residues, probably 1,4-linked.

The arrangement of the arabinose and galactose residues in the "hairy" fragments was studied after mild acid hydrolysis and enzymic degradation (Guillon and Thibault, 1989; Guillon et al., 1989). Hydrolysis by an arabinofuranosidase was a two step process where first α -1,3 bonds were splitted leaving a linear α -1,5 arabinan which is then hydrolysed. An endo-arabinanase, hydrolyzing preferentially linear arabinan, shows low activity on the initial "hairy" fragments but its activity is enhanced when the "hairy" fragments were incubated first or at the same time with arabinofuranosidase. The fact that β -galactosidase and endo-galactanase degrade only the "hairy" fragments, and that the galactanase activity was not increased by removing arabinose units, suggests that the galactanase is not hindered by arabinose side residues and that galactose units occur rather as short chains. This is confirmed by the high value of the ratio of

terminal to 1,4-linked galactose and by the absence of oligomers of galactose in the products resulting from a mild acid hydrolysis. More drastic treatment such as 0.1M trifluoroacetic acid (TFA) at 100°C for 1h did not remove all the galactosyl residues, suggesting the attachment of some galactose residues to galacturonic acid residues. Side-chains are therefore composed mainly of highly branched α -1,5-linked arabinans, the C₃ of the arabinosyl residues being the branching points, of β -1,4-linked galactans with few branching points on the C₃, and of β -1,3-, β -1,6-linked galactans.

We also investigated the location of the feruloyl groups. Part (30%) are ester-linked to arabinose units as shown by the fact that they are removed together with arabinose after mild acid hydrolysis with 0.05M TFA or after degradation with endo-arabinanase and arabinofuranosidase. The arabinose-ferulic acid compounds were not characterized, because of their low concentration. Possibly, feruloyl groups are located at the non-reducing arabinopyranosyl termini as described for spinach pectins (Fry, 1983). A subsequent part (~10%) of the feruloyl groups is released with galactose by the endo-galactanase, but a substantial amount (~30%) is obtained by a more severe acidic treatment such as 0.1M TFA. The remaining part is probably linked to some residual galactosyl units. The results suggest that all the feruloyl groups are not in equally exposed domains of the pectins and therefore, are not equally accessible (Thibault, 1988). A possible scheme of the structure of beet pectins is shown in Figure 2.

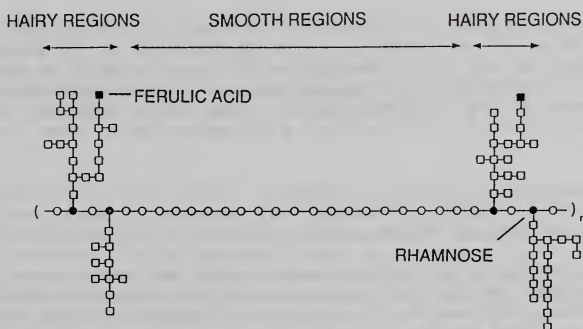


Figure 2. Schematic structure of sugar-beet pectins

EFFECT OF OXIDISING AGENTS

Results have clearly shown that the oxidants have not all the same effects on beet pectins (Table 2).

TABLE 2.--Action of some oxidants on sugar-beet pectins

Oxidant	concentration	changes in viscosity after 400 min
KIO ₃	10 mM	- 1
KMnO ₄	1 mM	-13
NaClO ₂	10 mM	-14
K ₃ Fe(CN) ₆	10 mM	- 5
H ₂ O ₂	0.1 mM	- 6
H ₂ O ₂ + peroxidase	0.1 mM + 1.3 mg/L	+36
(NH ₄) ₂ S ₂ O ₈	10 mM	+63

The addition of oxidants such as potassium periodate, potassium permanganate, sodium chlorite, potassium ferricyanide and hydrogen peroxide, to a solution of sugar-beet pectin leads to a continuous decrease of the reduced viscosity. In contrast, the agents hydrogen peroxide-peroxidase, and ammonium persulfate increase the viscosity of the medium. The former increases instantaneously the reduced viscosity to a value which decreases very slightly with time, whereas the latter causes a continuous increase of viscosity up to a maximum (Thibault and Rombouts, 1986).

Gels or solutions of markedly increased viscosity may be produced by addition of hydrogen peroxide-peroxidase or of persulfate, depending on the experimental conditions (mainly pH and concentration in pectin or in reagents) and also on the chemical structure of the initial pectin. All these aspects, including the mechanism of the reaction with persulfate ions, have been studied.

Participation of feruloyl groups. Three sets of results have been obtained which indicate that feruloyl groups are fundamentally involved in the reaction (Thibault and Rombouts, 1986):

(i) the contents of galacturonic acids and neutral sugars were not changed during the reaction. In contrast, marked changes in the content of feruloyl groups occur simultaneously with changes in the reduced viscosity, after an induction period. The content of feruloyl groups decreases continuously, whereas the reduced viscosity increased to a maximum value and then decreases slowly (Figure 3). The increase in viscosity is clearly due to a

polymerization process but the decrease is difficult to explain, since there is a complex dependence with concentration, molecular weight and shape of the polymers, pH and ionic strength which can all vary with the time of reaction;

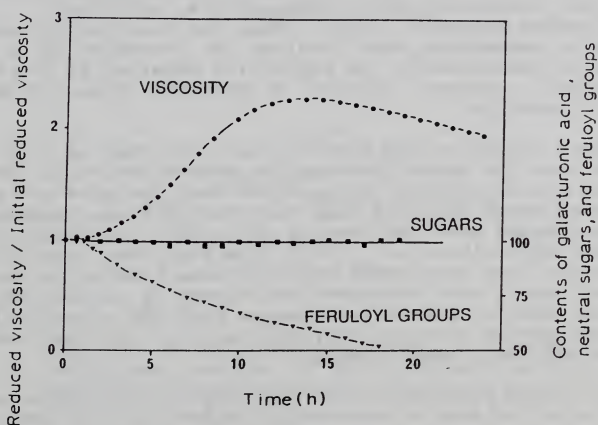


Figure 3. Changes with time of the viscosity, and the contents of galacturonic acid, neutral sugars and feruloyl groups of beet pectin in ammonium persulfate

(ii) the absorption spectra of the pectin under alkaline conditions show a decrease with reaction time of the peak at 375 nm and all the curves pass through an isosbestic point at 330 nm. This observation confirms that feruloyl groups specifically participate in the reaction;

(iii) gel-permeation chromatography on Sepharose CL-2B of the pectins shows an increasing amount of material rich in galacturonic acid and in neutral sugars eluting in the void volume where the feruloyl groups also accumulate. At K_{av} 0.85, a peak is left from which feruloylated material progressively disappears. This result proves that the polymerization is

specific for feruloylated pectins and that some of the pectin molecules are not feruloylated.

Mechanism of reaction. Sugar-beet pectins can therefore be crosslinked by the action of oxidising agents such as hydrogen peroxide/peroxidase or persulfate, which are known to create free radicals. Hydrogen peroxide alone acts only as a pure oxidising agent whereas the addition of peroxidase leads to the formation of free radicals. The persulfate ions decompose in aqueous solution to give radicals and are used in the presence of different reducing agents or alone for aqueous polymerization of acrylamide, methacrylamide, acrylonitrile, or methylmethacrylamide (Thomson, 1983). That the reaction of sugar-beet pectin with persulfate ions involves the formation of free radicals is confirmed by the inhibition observed by the addition of 1-propanol, acetate, citrate, or phosphate ions, which scavenge free radicals.

The radical nature of the reaction was confirmed by kinetic data obtained from the spectrophotometric determination of the feruloyl ester groups, taking into account that the products absorbed at the wavelength used and that the reactions are complete. The same experiments were carried out on ferulic acid taken as a reference (Thibault et al., 1987); the fact that ferulic acid alone reacts with persulfate with the same kinetic parameters (reaction order, rate constants and energy of activation) as sugar-beet pectins strongly indicates that the crosslinking of sugar-beet pectin involves only the feruloyl residues and not proteins which are also present.

The reaction of persulfate ions with sugar-beet pectin (or ferulic acid) follows a pseudo-first order law with respect to pectin (or ferulate) and not a pseudo-second order law (Figure 4). This result shows that the reaction with persulfate is not an intermolecular condensation of feruloyl residues (which would lead to a second-order reaction) as suggested for feruloylated arabinoxylan treated with hydrogen peroxide/peroxidase (Geissmann and Neukom, 1973).

¹H.n.m.r. studies on ferulic acid demonstrate that the aromatic nuclei are not modified, but that the double bonds are involved in the reaction (Thibault et al., 1987). A broadening of the signals from aromatic protons was observed and ascribed to a polymerization process, and this inference accords with the gel-permeation chromatography data which indicated oligomers of ferulates, with degree of polymerization up to 10, were produced. This polymerization is also confirmed by the fact that the rate of disappearance of ferulic acid is proportional to the ferulic acid concentration and to the square-root of persulfate concentration, as in a classical free-radical polymerisation.

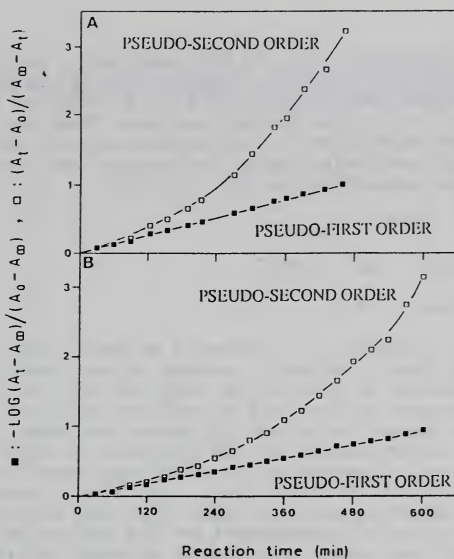
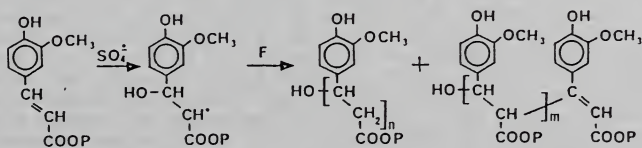


Figure 4. Kinetics of the action of ammonium persulfate on sugar-beet pectins (A) and on ferulic acid (B) analysed in term of pseudo-first and pseudo-second order reaction

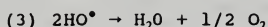
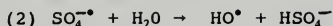
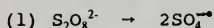
Therefore, the mechanism proposed for the reaction of persulfate ions with pectins and ferulic acid as shown in Figure 5 must apply. The polymerization of feruloyl groups in separate pectic molecules is probably sterically hindered and therefore polymerisation results mainly in the formation of dimers.



(F = FERULATE or FERULOYL; P = H or PECTIC CHAIN)

Figure 5. Possible mechanism for the crosslinking of beet pectins

The pH plays an important role in the reaction. Gelatin occurs only in the pH range, 3.8 - 5.7. Apparently, a low pH enhances the rate of disappearance of ferulic acid as well as feruloyl groups but not of the crosslinking reaction. This may be explained by the decomposition of persulfate ions to sulfate ion radicals that may react with water to produce the hydroxyl radical and oxygen according to Eqs. (1)-(3):



The reaction can therefore be initiated either by the sulfate ion radical or the hydroxyl radical. Low pH values favour non radical decomposition of persulfate ions and the increase of the rate of disappearance of feruloyl groups under acidic conditions can therefore be due to oxidation of these residues. However, analysis of the pH-dependence of the reaction is complicated because pectins are polyelectrolyte which can adopt an extended conformation in a fully ionized state, and a coil conformation in the acid form. Nevertheless, the fact that the rate constant for fully ionized pectins is independent of the nature of the counterion (sodium, calcium) and of the ionic strength of the reaction mixture and is not changed by enzymic depolymerization indicates that the conformation of the polymer does not play an important role in the reaction (Thibault et al., 1987). Apparently, the pH is the only factor that influences the rate of reaction and there must be a balance between a low pH which favour oxidation reactions, and neutral or alkaline pH, which may enhance reactions 2 and 3, leading to a more pronounced decay of the sulfate ion radical and to a rapid decomposition of the hydroxyl radical. On the other hand, pectic molecules can be slightly depolymerized by a β -elimination process under neutral conditions, even at 25°C. All these effects could explain the occurrence of gelation of pectins only in the pH range 3.8-5.7.

Properties of the products. Depending on the concentration of the reactants, the crosslinking reaction may be used to obtain pectins with increased molecular weight or to obtain gels with properties different from apple or citrus gels. The effect of crosslinking with hydrogen peroxide and peroxidase on viscosity-average molecular weight of the pectin is shown. It is seen that the molecular weight may be increased by 210% and that gels are obtained when the pectins are above a critical concentration (Rombouts et al., 1983). Striking increases in reduced viscosity can also be obtained with persulfate. As with the hydrogen peroxide/peroxidase systems, gels are obtained when the pectin concentrations above a certain value (Figure 6) (Thibault and Rombouts, 1986). A rheological study showed that the gels from

beet pectins are less brittle and more elastic than the gel prepared for a commercial amidated pectin (Rombouts et al., 1983).

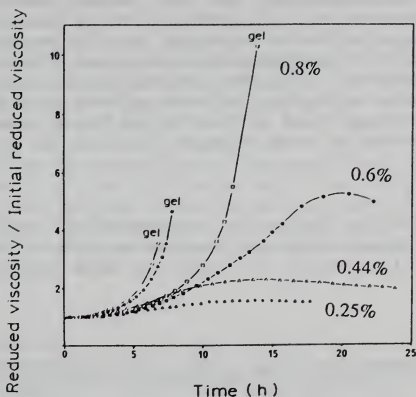


Figure 6. Changes of the viscosity of beet pectin solution at various concentration treated with ammonium persulfate

Another distinction is that beet pectin gels are irreversible as they are based on chemical links. The crosslinked pectins from such gels can therefore be isolated after a drying process. The products obtained do not dissolve in water but swell; they exhibit remarkable water-absorption capacities, e.g. 50-180 mL of water/g of product (Thibault, 1986). The crosslinking of sugar-beet pectins through their feruloyl residues yields a weak polyacid network. As the initial pectin contains less than 1% of feruloyl groups, the degree of crosslinking is low and the gels obtained from these products are highly swollen. The swelling depends on the degree of cross-linking, the degree of neutralization of the carboxyl groups, on the nature of the counterions, and on the ionic strength of the surrounding solution. All these variations can be explained by poly-electrolyte and Donnan effects. The acid forms of the crosslinked pectins swell to a rather limited extent (45-60 mL/g). When they are fully neutralized by sodium hydroxide, their bed volumes were multiplied by factors of 2 and 3 because the negatively charged groups of the network set up electrostatic repulsion which can expand the

network. The maximum value was ~180 mL/g (Table 2). The nature of the monovalent counterion (K^+ , Li^+ and Na^+) did not have a profound effect. A more limited swelling was observed when the samples were neutralised with calcium hydroxide because calcium ions are more tightly bound than sodium ions. However, shrinkages were not observed because of the low charge density of the beet pectins and because of the acetylation of the galacturonic acid residues. When the ionic strength was increased, the swelling of the modified pectins decreased and reached values observed in the acid form when the solution was 0.1M in NaCl (Table 3). This fact reflects the role of the screening of the fixed charges, resulting in a reduction of the electrostatic repulsion and therefore a reduction of the volume.

Table 3.--Effects of the degree of crosslinking, the nature of the counterion and the ionic strength of the medium on the swelling (mL/g) of modified beet pectins

Modified feruloyl residues (%)	74.6	74.1	70.0	69.4	67.1	66.7
<u>ionic form:</u>						
H ⁺	45	45	60	50	55	55
Ca ²⁺	80	75	100	75	100	125
Na ⁺	95	110	120	110	140	180
Na ⁺ in 0.001M NaCl	100	105	120	115	125	160
Na ⁺ in 0.01M NaCl	70	75	90	80	90	125
Na ⁺ in 0.1M NaCl	45	40	50	50	60	75

Influence of the structure of the side-chains on the reaction.

The study of the gelation of pectins extracted under different conditions from the pulp or from the cossettes led to the main conclusion that not all the pectins are able to form gels and that no simple relationship exists between the gelling capacity and the chemical composition of the pectins (Thibault, 1988). Therefore, the fine structure of the neutral side-chains of a pectin which was not able to gel, has been varied by acid and by enzymes in order to correlate the gelation to chemical changes in the pectic structure (Guillon and Thibault, 1987, 1990). The treatments included cold (25°C) or hot (100°C) dilute (0.05M) acid (TFA) hydrolysis and also hydrolysis by highly purified enzymes, namely arabinofuranosidase, endo-arabinanase, β -galactosidase and endo-galactanase. The results showed that pectins

capable of gelling were obtained only after cold acid hydrolysis or after the elimination of arabinose residues by the arabinofuranosidase. Structural analysis of the resulting pectins showed that the treatment with cold acid, as the treatment with arabinofuranosidase, leads mainly to a slight decrease of the terminal arabinose units while the other structural characteristics were not changed. In contrast, hydrolysis with β -galactosidase or extensive degradation of the arabinans by the combined action of arabinofuranosidase and endo-arabinanase or by hot dilute acid did not significantly improve the gelling power. Therefore, the conclusions seem justified that the feruloyl groups must be in exposed domains of the side-chains so that they are accessible and crosslinkable by persulfate ions and that the feruloyl groups in the periphery of the arabinose side-chains are of a special importance in the gelling process of beet pectins. In consequence, the chemical extraction of gelling pectins from sugar-beet must liberate these areas without extensive degradation of the arabinans.

CONCLUSION

Pectins are traditionally used as gelling agents and thickeners in the food industry. For these applications, pectins from apple pomace and citrus wastes are superior to the sugar-beet ones because they have higher molecular weight and they are not acetylated. We have reported the presence of feruloyl ester substituents in beet pectins and the possibilities for enzymic and chemical crosslinking through these substituents. This process may lead either to increased apparent molecular weight of soluble pectins, or to gel formation. The crosslinking reaction has now been studied considerable detail. In addition to the acid-sugar-pectin gel and the calcium-pectate gel, crosslinking is a third way of producing gels which is exclusive for pectins from sugar beet or other feruloylated pectins. The crosslinked pectins from these gels, isolated by drying have an extremely high water-absorption capacity. This property should lead to some new applications e.g. as a cloud stabiliser in drinks, or as water absorbing agent in sanitary products.

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DISCUSSION

Question: You have reawakened my interest in organic chemistry. When you were studying the cross linking reaction, did you look at the effect of iron II and hydrogen peroxide (The Fenton reaction) and did you look at free radical quenchers to kill the reaction?

Thibault: We have not studied the mixture of iron II and hydrogen peroxide. We did study several free radical quenchers, and they do inhibit the reaction.

Question: Thank you for a really interesting paper. The paper is doubly of interest to sugar processing, because ferulic acid is also involved in sugar colorants. You have identified ferulic acid, linked to the arabinose moiety. We had predicted that ferulic acid would be linked to a heteropolysaccharide group, and so carried into the factory, but had not been able yet to confirm that - thank you for doing so. Have you observed any increase in colorant when the polymerization reaction occurs?

Thibault: No, but that's probably because the sugar beet preparation is already dark.

Question: The previous question relates to another important area, the presence of high molecular weight colorants in sugar. Under what conditions are the ferulic acid groups removed from the polysaccharide?

Thibault: The ester linkages of ferulic groups are quite strong and require some acidic or alkaline conditions to hydrolyze - perhaps 2.0N NaOH for 1-2 hrs at room temperature.

THE DEVELOPMENT OF NEAR INFRARED (NIR) TECHNIQUE ON LINE IN THE SUGAR FACTORY

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INTRODUCTION

The remarkable development reached by the automatic control of plants in industry in general and in the chemical one in particular has obviously also involved the sugar industry. Although, particularly in some areas, the processing is carried out in a very short time, (50-60 days), and the highly variable raw matter entering the processing can change the composition of the relevant syrups, practically all the sections of a sugar factory are totally or partially automated.

The importance of this item in the sugar field is such that some years ago the Scientific Committee of the C.I.T.S. created a sub-committee for Measurement and Process Control which meets every year for an exchange of ideas about the continuous progress concerning this sector.

In Figure 1 and 2 we can see two examples of control benches which are completely automatic and computerized in two important sectors of a modern sugar factory processing.

However, the automatic management of the plants is meaningful not only if we know the physical parameters of the flowing syrups, but also the chemical ones which, as quoted above, are susceptible to appreciable variations. Here we have chemical control which should be not only reliable but able to give data very quickly which can be transmitted to the automatic control devices which manage the plants.

Chemical laboratories have in general followed the innovation process promoted by the electronics and computerization development and it is fairly usual to find in the sugar factory laboratories sophisticated apparatuses able to perform special analysis. Consequently, the problems are not related to the data collection and reliability but to the rapidity by which these data are obtained and made available for the automatic control of the plants. The operations carried out by the sugar factory laboratory, (Figure 3), that is sampling, analysis and data transfer, in practice give obsolete data to the automatic control system if they are not supplied in real time. In other words, if the data concern an emergency that occurred long before, the control device cannot obviously take care of the situation which has meanwhile changed. It is therefore more and more necessary

to employ instruments on-line which are able to give the highest number of data to the automatic control devices in real time.



Figure 1.--View of an automatic control bench - (Courtesy of CO.PRO.B Soc. a r.l.-Minerbio (BO), Italy).

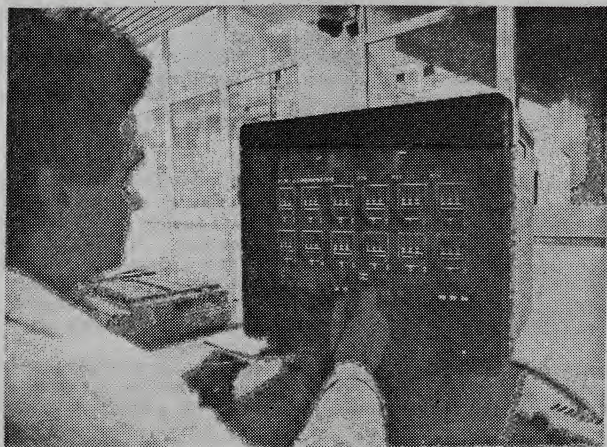


Figure 2.--Detail of a control monitor (cfr. Figure 1) - (Courtesy of CO.PRO.B Soc. a r.l - Minerbio (BO), Italy).

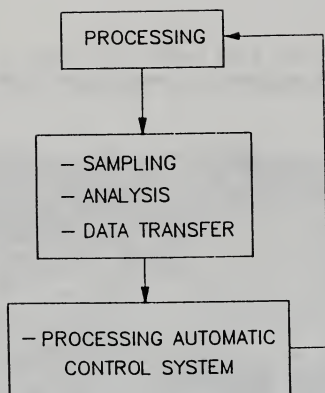


Figure 3.--Control traditional scheme

In our opinion, the NIR technique can be one of the ways for solving the problem of the on-line measurements. The NIR technique (Williams and Norris 1987) is simple based on the measurement of the amount of radiation absorbed by a sample, (which can be both liquid and solid), (Figure 4), which is appropriately irradiated with near infrared radiation (Figure 5). If the instrument has been properly calibrated, it can within a reading time of about 30 seconds, give a number of analytical data parameters concerning the sample that is the same as the calibrations related to its program.

The sample to be read, if liquid, is not in general subjected to any type of pretreatment or weighed but has only to be injected into the measurement cell. It is quite clear that such and non destructive measurement technique can very well be adopted by a control system on-line with direct transfer of data from the instrument computer to the automatic system which manages the functions of the different plants.

As far as the possibility of evaluation of different analytical parameters in the various types of solutions or products of the sugar processing is concerned, in recent years we have observed an increasing interest in the NIR technique. A lot of researchers have in general realized that this technique, although it is based on a simple empirical measurement of physical type, can give us sufficiently reliable analytical data of chemical type if suitable calibration curves have previously been set up.

In the special issue of the sugar magazine Zuckerindustrie (Vaccari and Mantovani 1989) printed in occasion of the XX I.S.S.C.T. congress we reviewed a number of papers concerning the

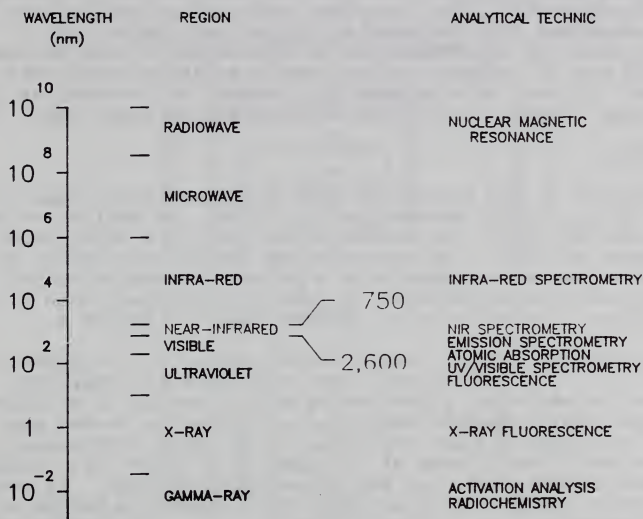


Figure 4.--Electromagnetic spectrum.

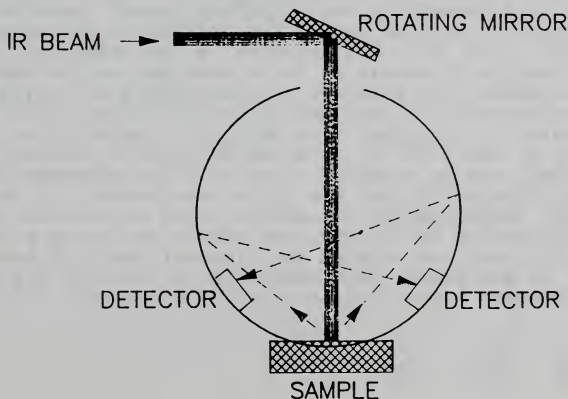


Figure 5.--Integrating sphere.

employment of the NIR technique in the sugar field. However, other papers have been published on the same subject (Berding *et al.*, 1989, Marchetti 1990), and just after this lecture this item will be presented and discussed by Nguyen and Player (1990). Moreover, although this technique is not an analytical methodology as such but it is based on previously existing analytical techniques, it will be discussed by a number of referees at the next session of the I.C.U.M.S.A. in Colorado Springs, USA.

EXPERIMENTAL

On the grounds of the results we have obtained in the laboratory (Vaccari *et al.*, 1987, Vaccari *et al.*, 1988), we decided to test the application of the NIR technique on-line in a sugar factory in the North of Italy. By employing an InfraLyzer 600D/S of the Bran+Luebbe Company equipped with a personal computer we set up a system of analysis in sequence of three juices (raw, thin and thick juices), according to the scheme shown in Figure 6.

The three juices, sampled from suitable points of the plants and cooled at 50° C, directly enter the sugar factory laboratory through stainless steel pipes having a diameter of 12 millimeters. With the purpose of obtaining a continuous stream and a constant piezometric load, the three juices flow continuously through a small tank placed at a height of 3 meters with respect to the measurement cell, and are directly discharged. By means of a suitable system of pneumatic valves, which are turned on and off by a computerizing system (Figure 7), the juices enter the thermostated (50° C) cell (Figure 8) in sequence, analyzed by the NIR apparatus (Figure 9), and are finally discharged. The three juices can also be manually sampled.

In Figure 10 the computerized system of turning on and off the valves is shown. After two minutes necessary for the cell cleansing (T01), two rapid ENTER-ENTER pulses are given for the reading and to transmit the data to the personal computer (T02); then, a reading is carried out by the instrument within 30 seconds (T03). The T02 - T03 sequence is then repeated two more times with the aim of obtaining three readings on the same juice. After this sequence the personal computer turns off valve no. 1 and turns on valve no. 2, and the operation sequence is repeated and the reading on juice no. 2 is obtained. After three readings on juice no. 2, valve no. 2 is turned off by the personal computer which then turns on valve no. 3 and all the sequence is repeated on juice no. 3, and the cycle starts again by opening valve no. 1. Consequently, a complete cycle of reading of the three juices require 20 minutes, and then a new cycle starts again. In this way, we have at our disposal every 20 minutes the same number of analytical data concerning a juice as the number of its calibration curves stored in the NIR device.

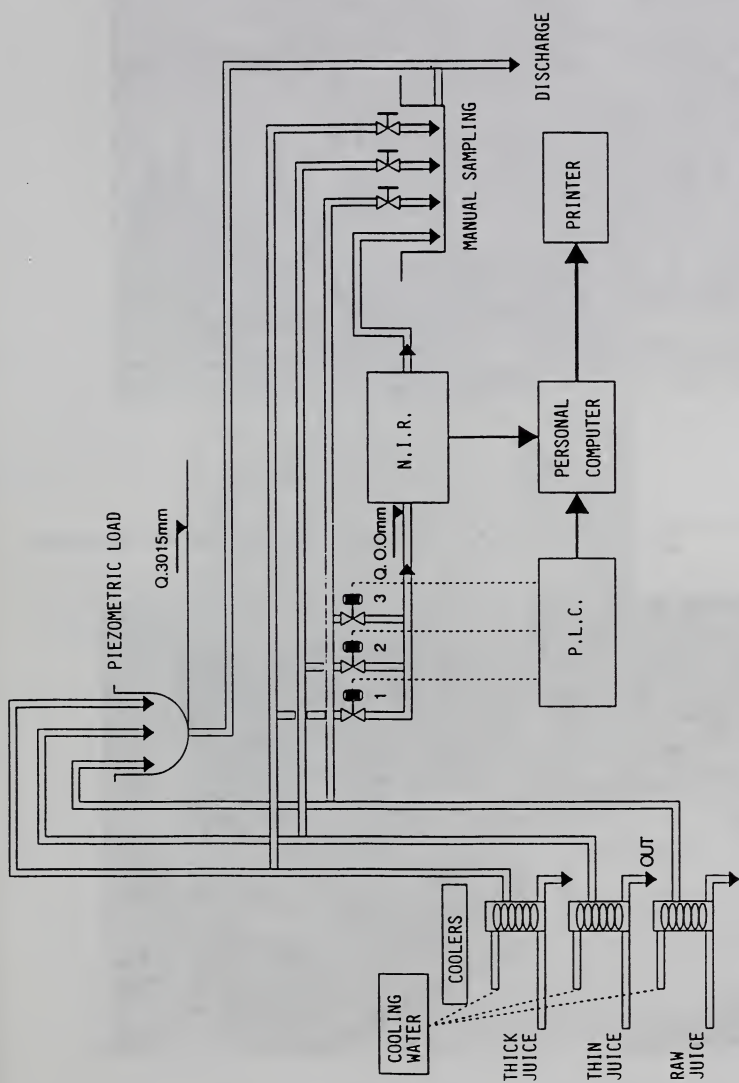


Figure 6.--Scheme of NIR on-line automatic system set up in the sugar factory of CO.PRO.B. Soc. a r.l. Minerbio, BO - Italy.

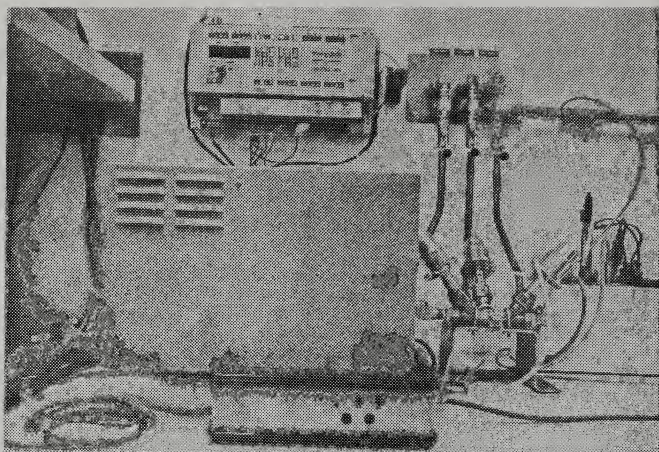


Figure 7.--PCL controlling the turn on and turn off of the three valves.

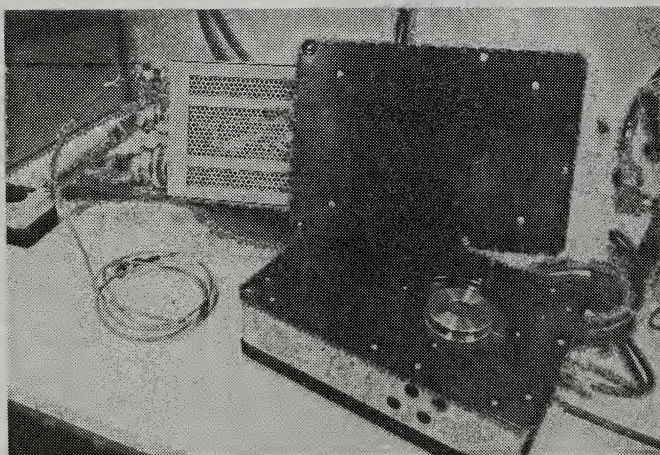


Figure 8.--The measuring cell (disassembled)

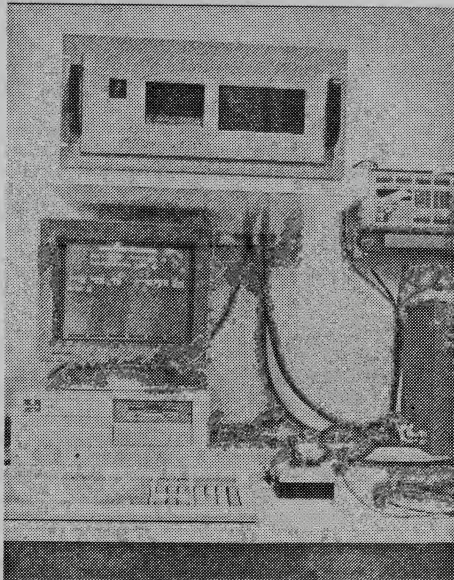


Figure 9.--NIR apparatus.

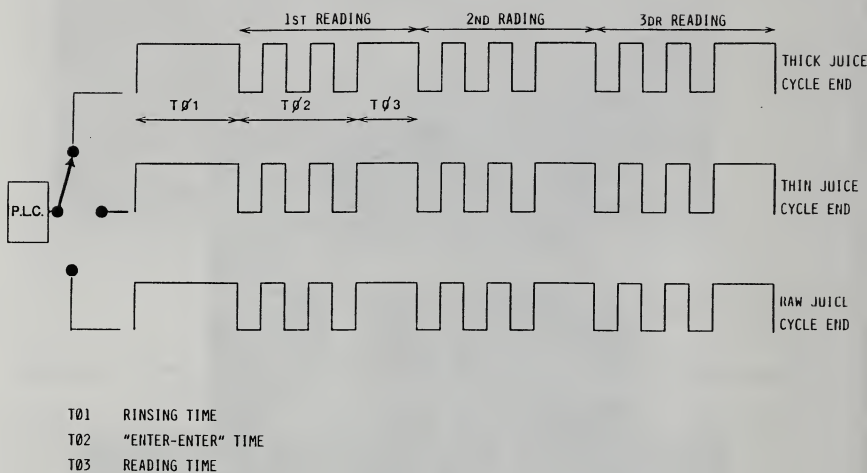


Figure 10.-- Computerized system of turning on and off the valves.

On the grounds of our previous laboratory experiments, we prepared during the last campaign the calibration curves for Brix and polarization but other parameters can be taken into consideration, such as, for instance, organic nitrogen as later described.

In tables 1 and 2 the statistical parameters related to the calibration curves obtained in the laboratory and following the on-line technique, are compared. The data can be considered sufficiently satisfying taking into account that the operation conditions during the on-line measurements were less favorable than the ones available in the laboratory. In fact, the continuous flowing of the juice unfavorably influences the measurements also bearing in mind that there was no automatic washing of the cell. Moreover, although we use the cooling devices described above, some variation of temperature and flow occurred. Whereas thin and thick juices were limpid, small beet particles were suspended in the raw juice which needed two filtration steps before entering the measuring cell which has a thickness of 0.5 mm and a gold bottom. The cell was not automatically rinsed, and it was necessary to wash it every now and then with hot diluted acid solution to eliminate possible light precipitates.

A general view of the measurement apparatus as a whole is shown in Figure 11.

With the purpose of examining the possibility of increasing the number of chemical parameters which can be evaluated by the NIR technique, we carried out laboratory experiments on the total organic nitrogen determination in raw juice.

Raw juice samples were analyzed during the whole season by Kjeldahl method and data were expressed as protein nitrogen. 45 samples were stored at -20°C and then utilized for the setting up of the relevant calibration curve by means of an InfraAnalyzer 450 according to the method described in previous papers.

The calibration curve related to two filters shows a correlation coefficient of 0.97 and a residual standard deviation of 0.025. By using this curve, 34 raw juice samples were analyzed and we compared the relevant data with the ones obtained by the Kjeldahl method, as shown in Figure 12. From the statistical data we can observe that there is no significant difference at 99% of probability between the two methods of measurement. In Figure 13 the frequency of distribution between the NIR and Kjeldahl values is shown: 50% of the differences are within ± 0.01 .

Table 1.--Polarization: parameters relating to the laboratory (Vaccari et al., 1987) and on-line prototype calibration curves.

	<u>RAW JUICE</u>				<u>THIN JUICE</u>				<u>THICK JUICE</u>			
	A	B	C	D	A	B	C	D	A	B	C	D
LABORATORY	36	2	.99	.12	--	-	---	---	44	2	.99	.46
ON-LINE	62	5	.99	.13	69	5	.99	.10	63	3	.98	.54

- A - Number of samples used for setting up of the calibration curves
 B - Number of filters
 C - Correlation coefficient
 D - Standard deviation

Table 2.--Brix: parameters relating to the laboratory (Vaccari et al., 1987) and on-line prototype calibration curves.

	<u>RAW JUICE</u>				<u>THIN JUICE</u>				<u>THICK JUICE</u>			
	A	B	C	D	A	B	C	D	A	B	C	D
LABORATORY	37	3	.99	.09	--	-	---	---	45	2	.99	.45
ON-LINE	61	5	.99	.14	68	4	.98	.14	62	2	.97	.68

- A - Number of samples used for the setting up of the calibration curves
 B - Number of filters
 C - Correlation coefficient
 D - Standard deviation

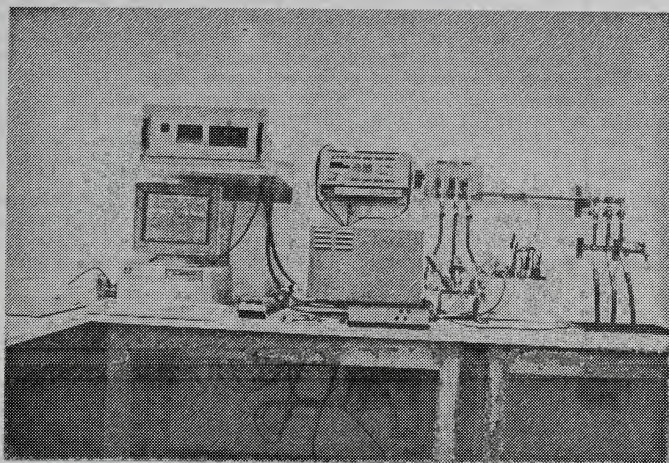


Figure 11.--General view of the measurement apparatus.

CONCLUSIONS

The results we obtained by using this first application prototype of the NIR technique on-line seem to be sufficiently encouraging although some problems remain to be solved and possible improvements could be made to the system as a whole.

It remains to be stated if and how the calibration curves set up last year to be modified during the next season. Moreover, the frequency of these possible modifications has to be verified, taking into account that, although encouraging, the laboratory results we have obtained need further confirmation.

As far as the improving of the results is concerned, this can be achieved by interrupting the flow through the measurement cell, by improving the thermostatzation and by adopting an automatic hot cleaning of the cell itself.

Besides these measures we plan to analyse by the on-line NIR technique some other juices of the sugar end which can reach the instrument through other suitable pipelines.

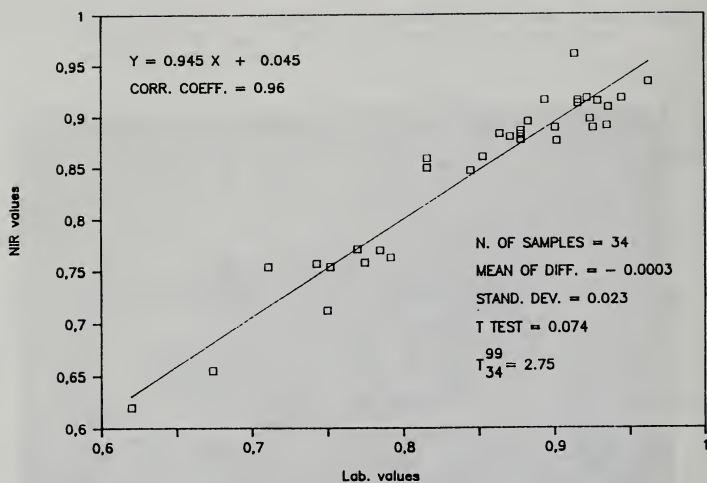


Figure 12.--Comparison between proteic nitrogen data obtained by NIR and Kjeldhal method.

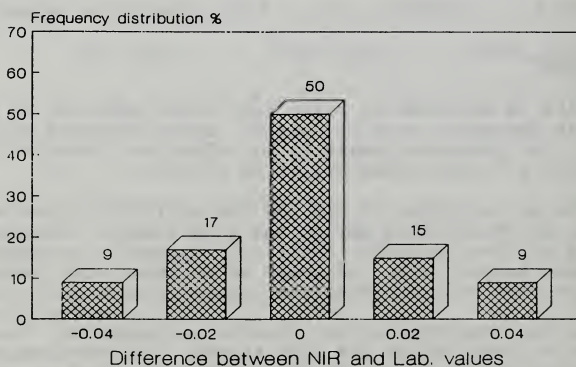


Figure 13.--Differences between proteic nitrogen data determined by NIR and Kjeldhal method.

Obviously, the increase of the number of juices to be analyzed by means of only one measurement cell decreases the frequency of the analysis of each juice. However, in view of the favorable results we could obtain during the next campaign, we can take into consideration the possibility of setting up a number of measurement cells in different points of the sugar factory. In this way it would be possible to collect all the data by a centralized unit which could be able to transmit them in actual time to the automation systems of the stations. Moreover, these cells could better fit the various types of juices, for instance by modifying their thickness, and would almost continuously give data since they would be fed by only one juice.

The possibility of increasing the number of chemical parameters to be measured on the various juices, such as for instance nitrogen, will allow us to obtain an even more complete picture of the operation of the plants and, consequently, take immediate steps.

ACKNOWLEDGMENTS

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DISCUSSION

Question: Thanks, Professor Vaccari, for your interesting presentation. I have two questions. First, how often do you have to make a calibration of your system; and second, have you had some problems with crystallization of sugar in your system?

Vaccari: The first question is related to the frequency of recalibration. During the last campaign, as I explained, we prepared calibration curves, and we intend to verify this in the next campaign. But during the last year we prepared in the laboratory (not on the on-line apparatus) a calibration curve using samples collected during the previous campaign. Then, in the following campaign, we used the same calibration curve to analyze samples coming from the same sugar factory, collected during the whole campaign, and the only operation we needed was to modify slightly the change in slope of the calibration curve.

Your second question is obviously related to the thick juice because we have no crystallization problem in raw juice or thin juice. In this prototype we use a thick juice from the exit of the 4th evaporator, because we want to avoid the problems of crystallization. The Brix of the thick juice was therefore not high enough to crystallize at 50°C. Obviously, if we wanted to analyze the more concentrated juices, we need to operate not at 50°C but at perhaps 80°C.

Question: In the pipelines used for the transport of your samples, do you ever observe microbial infections?

Vaccari: You are referring to the raw juice, obviously. We never noticed a problem in this field because the distance from the plant to the laboratory is not too long. We have not noticed an increase in parameters related to microbiological infection. We had no problems higher than the normal problems in the laboratory. We must take into account that we have a continuous flow of juice; we never stop the flow in the pipe, so that the continuous flow may prevent this kind of problem.

Question: One of the classic compounds that we are interested in is nitrogen; we can get a good estimate on that. Organic anions, in particular acetate, oxalate, citrate and lactate, and that whole group are a problem. Have you done calibration curves on organic anions?

Vaccari: We have no experience in a calibration curve for these kinds of compounds. We know that these compounds are present in low concentration, so that there could be some difficulties. We were skeptical also about the nitrogen content determination because the nitrogen content is very low, but we obtained good results. Possibly in the future we can consider analyzing other

organic compounds. I know that someone obtained a good calibration curve, for example for calcium salts, and for color.

Question: What effect have you noticed with entrained air in your samples?

Vaccari: Obviously if bubbles of air enter the cell, there will be a distorted signal. But in the different juices we use, the thick juice, thin juice and raw juice, we have had no problem with bubbles. Obviously, we could meet the problem of bubbles, in molasses for example. When we prepare a calibration curve in the laboratory using molasses, we dissolve the molasses in water (50%) to avoid this problem.

Question: Do you not have a lot of dissolved air in your raw juice?

Vaccari: We don't know the amount of dissolved air, but if there is more or less constant air dissolved, this parameter will be taken into account in preparing the calibration curve, because the matrix effect contains all of the parameters, such as turbidity, for example. The occurrence of other conditions not related to the parameters that we want to analyze, as for instance the presence of air, the variation of the temperature and so on, are compensated by the matrix effect.

Question: Do you see a continued evolution of the instrument design towards more accurate and precise measurements or do you judge that the NIR technique is precise enough to be applied to control solutions involved in sugar processing?

Vaccari: We need to have a system of analysis for correctly analyzing the sample so that we can prepare calibration curves, correlated with known results. Obviously, we cannot obtain more reliable results than we can obtain using the external systems of analysis because we use the results of that analysis to obtain a calibration curve. We can easily obtain results close enough to the external method using NIR. I think we can use NIR because of its advantages; we don't need to manipulate or clarify the sample; we have the results within a few seconds; we can send the resulting data directly to the computer at the plant.

NEAR INFRARED ANALYSIS OF CANE SUGAR PRODUCTS

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INTRODUCTION

Near infrared analytical (NIRA) methods are based on the empirical correlation between the NIR spectrum of a sample and its chemical composition. Absorbance in the near infrared region (750-2650 nm) corresponds to overtones and combinations from the vibrations of chemical bonds, particularly those involving hydrogen. Since a large number of organic compounds contain C-H, N-H and O-H bonds, NIR methods have found many applications in the agricultural, chemical and pharmaceutical industries.

The methods have the advantage of being fast, simple and non-destructive. Simultaneous analyses of several components can be carried out with minimal sample preparation. The samples may be in the form of solids, liquids or pastes. For solid and paste samples, reflectance methods are most often used whereas transmission or transreflectance methods are commonly employed for liquid samples. Granular solid samples are often ground to ensure homogeneity of crystal size as NIR absorbance is also influenced by the granularity of samples. However, in low moisture samples such as sugars, sample grinding prior to NIR analysis is not practicable because it may result in significant loss of moisture which could lead to analytical errors. Therefore, the calibration samples should have a range of crystal sizes randomly distributed with respect to and independent of the chemical composition of these samples in order to minimise the effect of grain size variation in the determination of constituent concentration.

Many applications of NIRA methods for the measurement of sugars in various agricultural products have been reported. For example, McClure et al., (1977) developed NIRA calibration for total reducing sugars in tobacco leaf. Dull et al., (1978) investigated the measurement of individual sugars in fresh fruit tissue by NIR analysis, finding that the strong water absorption band makes the determination of sugars difficult. Giangiacomo et al., (1981) studied the measurement of individual sugars in dried apples and apple juices by NIRA. Analysis of juices for individual sugars was found to be unsatisfactory. Later, these authors reported the results of NIR analyses of artificial mixtures of sucrose, glucose and fructose in aqueous solutions. Standard errors of prediction between 0.35 and 0.69% were reported for the measurement of individual sugars (Dull & Giangiacomo, 1984; Giangiacomo & Dull, 1986). Lanza and Li (1984) used NIR spectroscopy for the analysis of total sugars in eleven types of

fruit juices but found accuracy and precision of the determinations to be unacceptable.

The potential of NIRA methods in cane and beet sugars was first studied by Curtin (1986) and Vaccari et al., (1987) respectively. Curtin's study was concentrated on NIRA as a laboratory method for the determination of chemical constituents in cane raw sugars. Vaccari's study involved beet samples and process streams such as juices and molasses in beet sugar factories.

Curtin's preliminary study was followed by a more detailed investigation (Ames et al., (1989)) involving a large calibration set over 600 cane raw sugar samples and a scanning near infrared spectrophotometer to investigate all the wavelengths in the near infrared range. This study found that calibrations using all available wavelengths improved the prediction accuracy only marginally in most cases over the calibrations based on nineteen filter wavelengths (Table 1).

Prediction accuracy of around 0.110 units for pol and 0.030 units for water was judged to be acceptable for process control but not good enough for the payment of cane or sugar. Vaccari et al., (1987) using a 19 filter Technicon instrument equipped with a transreflectance cell reported satisfactory results for the NIRA measurement of dry substance and sucrose content in beet brei, raw juice, thick juice and molasses. Molasses was diluted 1:1 before measurement. Prediction errors for dry substance determination averaged 0.178 units in juice and 0.267 units in molasses. Sucrose content prediction errors averaged 0.277 units in beet brei, 0.140 units in raw juice, around 0.233 units in thick juice, and 0.279 units in molasses. There was generally good transferability of calibrations between factories except for one incidence where abnormally high errors (over 1.0%) were found in the dry substance measurement using the calibration developed at another factory. The same calibrations were also used in the following year in the factories to test their stability. Similar prediction accuracy was obtained in the second year indicating there had been no change after one year (Vaccari et al., 1988).

Another study concerning beet molasses was carried out by Dumoulin et al., (1987) who used a 19-filter NIR instrument in the transreflectance mode to monitor the total sugar and ethanol concentrations during fermentation of a molasses broth. Prediction errors of 0.35% for sugar and 0.12% for ethanol were reported. Sverzut et al., (1987) employed a scanning near infrared spectrometer in reflectance mode to determine the pol, fibre, sucrose and moisture content of shredded sugarcane samples. Standard errors of prediction for these measurements were reported to be 0.567% for pol, 1.064% for fibre, 0.540% for sucrose and 0.983% for moisture. The authors used the second derivative of absorbance values ($\log 1/R$) in data analysis instead of absorbance values directly because it is claimed to minimize the

effect of variability in particle size. This claim appears to be substantiated in this study as the multiple correlations of between 0.84 and 0.96 obtained with the second derivatives were significantly better than those obtained with log (1/R) (between 0.70 and 0.80). However, data analysis for raw sugars in our study using second derivatives showed no such improvement over log (1/R) (Table 2).

TABLE 1.--Comparison of NIRA calibrations for raw sugar based on a selection of filters or all wavelength combinations

Parameter		Multiple correlation (R)	Standard errors of prediction (SEP)
Pol/	3 wavelengths	0.981	0.107
	3 filters	0.978	0.118
Water/	2 wavelengths	0.985	0.030
	2 filters	0.984	0.031
Reducing sugars	3 wavelengths	0.910	0.054
	3 filters	0.906	0.055
Ash/	3 wavelengths	0.951	0.035
	3 filters	0.934	0.040

TABLE 2.--Comparison between NIRA calibrations for raw sugar samples based on log (1/R) and its second derivative

Parameter	Number of wavelengths	Multiple correlation coefficient	
		log 1/R	2nd derivatives
Pol	2	0.974	0.975
	3	0.981	0.976
	4	0.982	0.978
Water	2	0.984	0.982
	3	0.986	0.983
	4	0.986	0.984
Reducing sugars	2	0.932	0.880
	3	0.934	0.884
	4	0.936	0.887
Ash	2	0.934	0.931
	3	0.948	0.946
	4	0.955	0.949

Berding et al., (1989) using near infrared reflectance to analyse the fibre, moisture and sugar content of fibrated cane and the brix, pol, purity in cane juice. Correlation values of 0.924, 0.924 and 0.912 were obtained for fibre, moisture and sugar content, respectively, in fibrated cane. The corresponding errors of prediction were 0.586%, 0.569% and 0.524%. For pol measurement in expressed juice, the correlation was 0.975 and the prediction error was 1.335%. This substantially higher than the prediction errors found in beet juice reported earlier by Vaccari et al. (1987, 1988).)

INSTRUMENTS AND METHODS

Near Infrared Analysis

The near infrared spectrometer employed in this study was a Technicon (now Bran & Lubbe) InfraAlyser 500A. This is a scanning monochromator which can scan over the range 600-2500 nm. The scanning rate of this instrument is very slow at about 90 seconds per scan, compared to some later instruments such as the NIR Systems 6500 or the Quantum 1200 which are capable of 2-5 scans per second. The advantage of a faster scanning rate is that many scans can be averaged (typically 50) to minimise the variability caused by instrument noise. This may be important in the analysis of liquid samples especially in on-line applications or transmittance. Due to the slow scanning rate of our instrument, scan averaging was not carried out. However, each sample was presented 4 times. The average of these scans were used in data analysis.

Two sampling devices were tested for liquid samples: a molasses cup and a liquid drawer. The molasses cup is a small cell in which one or two drops of molasses are put, the glass cover is then screwed on, forming a thin film of molasses 0.3 mm thick between the glass surface and a gold-plated back plate. The liquid drawer is a jacketed device into which samples can be injected which form a thin film similar to that in the molasses cup. The injection feature of the liquid drawer is an advantage over the molasses cup in that it is not necessary to dismantle it to scan a new presentation of a sample. Both sampling devices operate under the transmittance mode in which part of the radiation is reflected at the liquid surface and part penetrates deeper into the liquid before being reflected.

Molasses samples were analysed directly at 70°C (undiluted) or at 25°C after 1:1 dilution. For analysis at 70°C, the molasses samples were warmed up in a microwave oven to about 45-50°C so that they were fluid enough to be injected with a syringe. It is important to avoid the entrapment of air bubbles in the liquid film being scanned as this introduces large errors in the NIRA results.

Data Analysis

Spectral data were analysed by either multilinear regression or principal components methods using the Bran & Lubbe IDAS software. Values of $\log I/R$ or its first and second derivatives were used in these analyses for comparison and to select the best wavelengths.

Reference Analyses

For raw sugars, ICUMSA methods of analysis were employed. The measurement of individual sugars in molasses was by HPLC with BioRad HPX-87N column. For the determination of ethanol and glycerol in fermentation broth, the BioRad HPX-87X column was used. Total sugars in molasses was determined by acid inversion followed by constant volume Lane and Eynon titration. Sucrose in molasses was determined by double polarisation method. Fermentation yield of molasses was measured by a method used by CSR Distilleries.

RESULTS AND DISCUSSION

Raw Sugars

Table 3 summarises the results for the measurements of chemical and physical parameters in cane sugars. The best results were obtained for water determination but reasonably accurate predictions of pol, ash and reducing sugars were also obtained. Measurements of grain size by NIRA were not very accurate judging from the calibration statistics. However, it was found that useful prediction of percent fines in the factory could be obtained by NIRA. In general, NIRA predictions in the factory can be substantially more accurate than that indicated by the standard errors of the overall calibrations which are based on a large number of samples from many different growing areas. For example, the standard error in the overall calibration for pol in raw sugars is 0.107 compared to 0.061 standard error in the prediction of samples from a single mill (Victoria).

On the other hand, it has been observed that frequent bias adjustment may be required to correct for periodic drifts, particularly in pol measurement (Ames et al., 1989). These adjustments may be due to systematic bias caused by unknown seasonal factors or changes in particle size. Although a large number of samples had been included in the calibration set, apparently some of these variabilities still existed. Figures 1 and 2 show the correlation between NIRA prediction and actual values for pol and moisture in raw sugar. These figures are illustrative of the best correlations that were obtained for raw sugar. Figure 3 shows the correlation for ash which is not nearly as good as that for pol and water.

TABLE 3.--NIRA calibration and prediction for cane raw sugars

Parameter	Calibration method	R	SEP
Pol	3 wavelengths	0.981	0.107
	3 filters	0.978	0.118
Water	2 wavelengths	0.985	0.030
	2 filters	0.984	0.031
Reducing Sugars	3 wavelengths	0.910	0.054
	3 filters	0.906	0.055
Ash	3 wavelengths	0.951	0.035
	3 filters	0.934	0.040
Percent Fines	3 wavelengths	0.784	3.9
	3 filters	0.769	3.7
Mean Aperture	3 wavelengths	0.805	0.044 (mm)
	3 filters	0.774	0.047
Mean Elongation	3 wavelengths	0.719	0.08
	3 filters	0.712	0.08

Soft Brown Sugars

The results of NIRA calibrations for soft brown sugars are summarised in Table 4. Figures 4, 5 and 6 illustrate the correlation between predicted and actual values for water, reducing sugars and sucrose. The rather poor result for reducing sugars is common to all products. In general, the calibration multiple correlations for soft brown sugars are similar to those for raw sugars but prediction error was significantly higher. This may be due to the presence of interfering impurities in the former products which contain up to 8% impurities compared to less than 2% in raw sugars. For soft brown sugars it is also notable that much greater improvement was obtained with calibrations based on all wavelengths over the filter-based calibrations than was the case with raw sugars.

TABLE 4.--NIRA calibration and prediction for soft brown sugars

Parameter	Calibration method	R	SEP
Sucrose	3 wavelengths	0.974	0.193
	3 filters	0.933	0.315
Water	2 wavelengths	0.995	0.038
	2 filters	0.994	0.042
Reducing Sugars	3 wavelengths	0.918	0.135
	3 filters	0.885	0.158
Ash	3 wavelengths	0.949	0.051
	3 filters	0.930	0.060

Liquid Mixture of Sucrose, Glucose, Fructose

We repeated the experiment carried out by Giangiacomo & Dull (1986) in which artificial mixtures of sucrose, glucose and fructose were analysed by NIRA using a NEOTEC instrument in the transmittance mode. With the Bran & Lubbe 500 in the trans-flectance mode, broadly similar prediction accuracy was obtained (Table 5). This experiment suggested that there may be a theoretical limit in the accuracy of NIR analysis of about 0.5% SEP for individual sugars in aqueous mixtures of sucrose and reducing sugars even when there is no interference from other impurities and minimal errors in the value of actual sugar concentrations. Since there are significant levels of both glucose and fructose in cane molasses, NIRA measurement of sucrose in these products may be adversely affected and therefore significantly less accurate than in beet molasses which contain relatively low levels of reducing sugars.

TABLE 5.--NIRA calibration and prediction of aqueous sugar mixture

Parameter	Calibration method	R	SEP
40% mixture			
Sucrose	2 wavelengths	0.999	0.53
Glucose	2 wavelengths	0.999	0.67
Fructose	2 wavelengths	0.998	0.84
20% mixture			
Sucrose	2 wavelengths	0.996	0.49
Glucose	2 wavelengths	0.994	0.60
Fructose	2 wavelengths	0.994	0.63
Combined 20% & 40% mixture			
	2 wavelengths	0.995	1.01
	2 wavelengths	0.997	0.80
	2 wavelengths	0.996	0.93

Mill Molasses

A selection of forty-five C-molasses samples from various CSR mills was used in the calibration for sucrose, total sugars, water, reducing sugars, ash and fermentation yield. Another twenty samples were used to verify these calibrations. The samples were analysed by NIRA using three different methods of sample preparation.

- (1) Liquid drawer, 70°C undiluted
- (2) Liquid drawer, 25°C, diluted 1:1 with water
- (3) Molasses cup, room temperature, undiluted

A summary of the various calibration and prediction statistics is presented in Table 6. While the molasses cup appeared to give the best results for total sugars prediction, the liquid drawer (methods 1 and 2) sometimes gave better results for other variables. There was no consistent trend which clearly indicated the superiority of any one method over the other. In terms of convenience and ease of operation, method (1) was the preferred method.

TABLE 6.--NIRA calibration and prediction of mill molasses

Parameter	Calibration method	(1)		(2)		(3)	
		R	SEP	R	SEP	R	SEP
Total Sugars	3 wavelengths	0.952	0.55	0.963	0.48	0.972	0.42
	3 filters	0.940	0.60	0.940	0.56	0.945	0.58
Sucrose	3 wavelengths	0.811	1.03	0.820	0.82	0.878	0.69
	3 filters	0.788	1.08	0.805	0.97	0.789	0.89
Water	2 wavelengths	0.949	0.34	0.855	0.56	0.940	0.37
	2 filters	0.926	0.41	0.791	0.66	0.910	0.45
Reducing Sugars	3 wavelengths	0.951	0.57	0.916	0.74	0.942	0.62
	3 filters	0.938	0.64	0.898	0.81	0.937	0.64
Ash	3 wavelengths	0.856	0.75	0.960	0.40	0.950	0.45
	3 filters	0.846	0.76	0.950	0.45	0.942	0.48
Fermentation Yield	3 wavelengths	0.798	1.02	0.824	0.97	0.840	0.96
	3 filters	0.795	1.03	0.803	1.01	0.796	1.03

(1) Liquid drawer, 70° C, undiluted

(2) Liquid drawer, 25° C, diluted 1:1

(3) Molasses cup, room temperature, undiluted

Significant gains in calibration correlation and prediction accuracy were obtained when all wavelengths in the near infrared range were considered rather than just the nineteen filters. These gains are larger than those found with raw sugar (Table 1). This appears to be true for all liquid samples investigated in our study and probably suggests that scanning NIR instruments are required for the analysis of liquid products.

Figures 7 and 8 show the correlation between NIRA prediction and reference results for sucrose by double polarisation and dry substance of C-molasses. The prediction errors in sucrose and water determinations are 2-3 times those reported for beet molasses. This may be due to the more complex nature of cane molasses and higher impurity levels, especially high levels of reducing sugars.

Refinery Molasses

For the investigation of refinery BBO syrup, forty-five samples were included in the calibration set and another twenty samples were used in the verification set. The samples were analysed undiluted by the liquid drawer at 70°C. Most BBO samples were fluid enough to be injected by syringe directly at room temperature and did not require warming like the C-molasses. Results of calibration and prediction are presented in Table 7 and Figures 9 and 10 illustrate the correlation for dry substance and reducing sugars. Overall prediction accuracy is disappointing compared to C-molasses. This is especially so considering that the purity level in BBO samples was significantly higher than in C-molasses and similar to that of beet molasses. One possible explanation is the relatively high levels of reducing sugars in BBO syrups (approx 20% of total sugars) which may have an adverse effect of NIR analysis of sucrose, although this does not explain the poor accuracy of water predictions in these samples. Nor does it explain the better prediction accuracy in C-molasses which contain even higher RS levels than BBO samples.

TABLE 7.--NIRA calibration and prediction for refinery molasses

Parameter	Calibration method	R	Standard errors
Total Sugars	3 wavelengths 3 filters	0.887 0.893	1.35 1.32
Water	2 wavelengths 2 filters	0.987 0.988	0.61 0.57
Reducing Sugars	3 wavelengths 3 filters	0.781 0.7575	0.68 0.71
Ash	3 wavelengths 3 filters	0.948 0.963	0.27 0.23

Distillery Fermentation Broth

Fifty fermentation broth samples were used in the calibration for water, total sugars, ethanol and glycerol by transfectance near infrared analysis. Samples were analysed at room temperature and prediction verification was carried out on twenty other samples. The results are summarised in Table 8 and typical correlations are shown for dry substance and ethanol in Figures 11 and 12.

The best results were obtained with water, dry substance, ethanol and ash. In comparison, NIR prediction of sugars and glycerol were less successful. Interestingly our results were also less accurate than those reported earlier for beet fermentation broth (SEP = 0.35 for sugars and 0.12 for ethanol) (Dumoulin et al., 1987). Once again, it appears that NIR analysis of cane sugar products may not be as successful as that of beet products, perhaps due to interference from higher levels of impurities in cane products.

TABLE 8.--NIRA calibration and prediction for distillery fermentation broth

Parameter	Calibration method	R	Standard errors
Total	3 wavelengths	0.885	1.27
Sugars	3 filters	0.894	1.22
Dry	3 wavelengths	0.997	0.65
Substance	3 filter	0.996	0.70
Ethanol	3 wavelengths	0.991	0.40
	3 filters	0.993	0.36
Glycerol	3 wavelengths	0.808	0.76
	3 filters	0.794	0.79
Ash	3 wavelengths	0.982	0.60
	3 filters	0.983	0.59

CONCLUSIONS

Our company as operators of sugar mills, refineries and distilleries has a significant involvement in chemical analysis. The early study by Curtin employed a nineteen filter instrument which was kindly lent to us by a instrument supplier. We were sufficiently encouraged by those early results to purchase the Infra-Alyser 500 to explore what at that time was the full potential of the NIR technique. We did not have a clear goal rather we wished to determine whether a better result could be obtained using wavelengths different from the nineteen available in the first instrument tested. Depending on the quality of prediction we expected to be able to recommend to our factories, areas where NIR analysis might profitably replace existing methods.

At this time, we have not recommended any NIR method to replace a currently used analytical method. Generally the reason for this is that the error of prediction is too high compared with the present analytical method. What criteria would we use to recommend an NIR method? There is probably no simple answer to this question because factors like the time to obtain a result and cost of analysis vary considerably among traditional analytical methods. With the present high cost of NIR equipment, NIR analysis is hardly economical unless a number of analyses can be performed on the same sample.

Obviously, the methods that are the most time consuming and costly to perform would be the best candidates for replacement by NIR analysis if the error of prediction were satisfactory. These would include sucrose by double polarisation, grain size determination, fermentation yield particularly. Unfortunately, the prediction errors for these analysis by NIR are too poor to be acceptable. To be acceptable, we believe that the error by NIR should not be greater than say 1.5 times that by conventional analysis. For example, we believe that the within laboratory standard error for double polarisation of a molasses sample is about 0.5. An NIR standard error of prediction of 0.8-1.0 is greater than the 1.5 criterion.

Another difficulty we have in recommending NIR methods is that we are suspicious about unknown samples being of the same population as those used to obtain calibration. This means that one can never completely give up the conventional analytical method used to calibrate the instrument.

At present, we are looking for opportunities to employ on-line NIR for process control. We have just done some testing with on-line instruments in transmittance mode and are encouraged about the possibility of measuring sucrose and water in solutions like clarified juice or mill liquor well enough to justify some process control applications. We believe the use of NIR methods for the payment of cane or sugar is still unrealistic.

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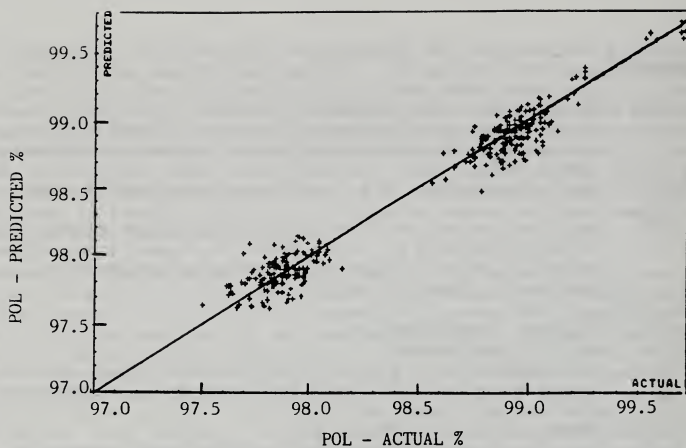


Figure 1. NIR prediction of pol in raw sugar using a 4 filter calibration

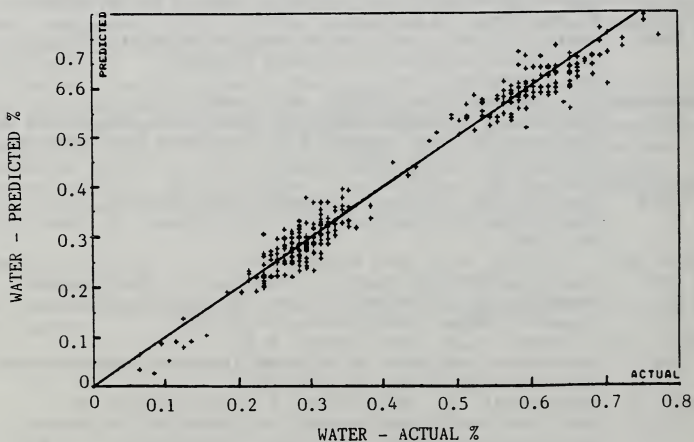


Figure 2. Prediction of moisture in raw sugar using a 2 filter calibration

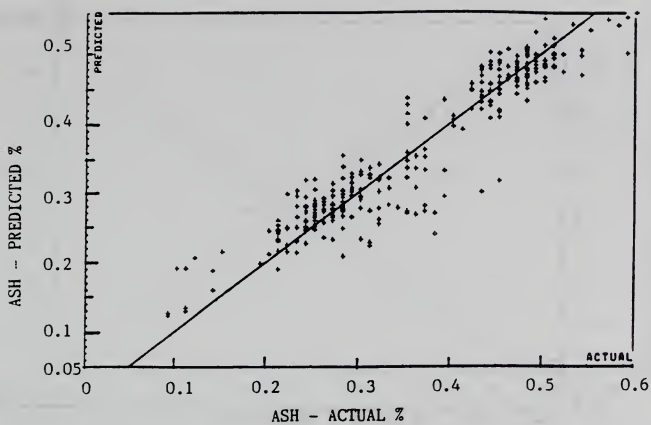


Figure 3. NIR correlation of ash in raw sugar by a 4 filter calibration

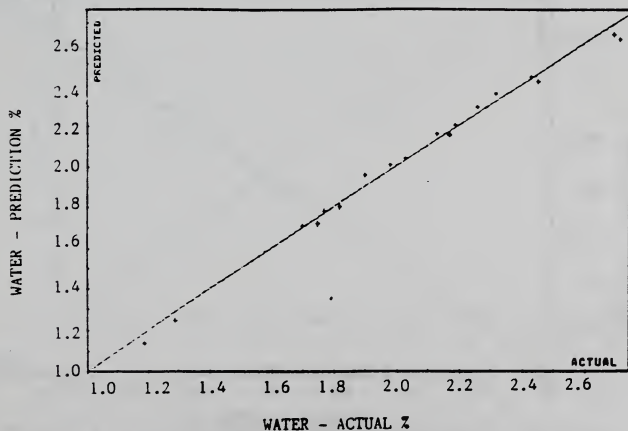


Figure 4. Prediction of water in soft brown sugar by NIRA

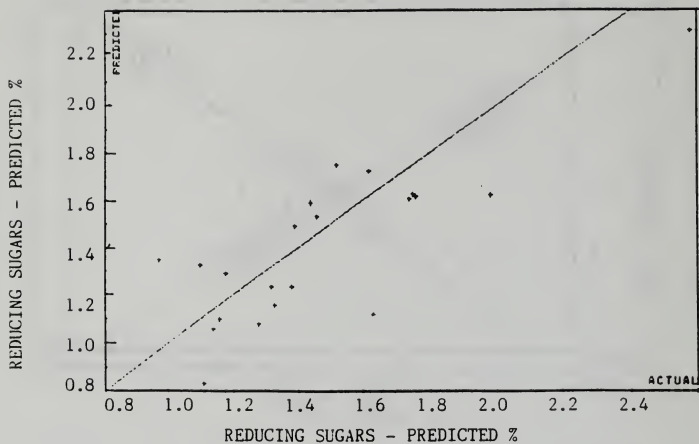


Figure 5. NIR correlation of reducing sugars in soft brown sugar

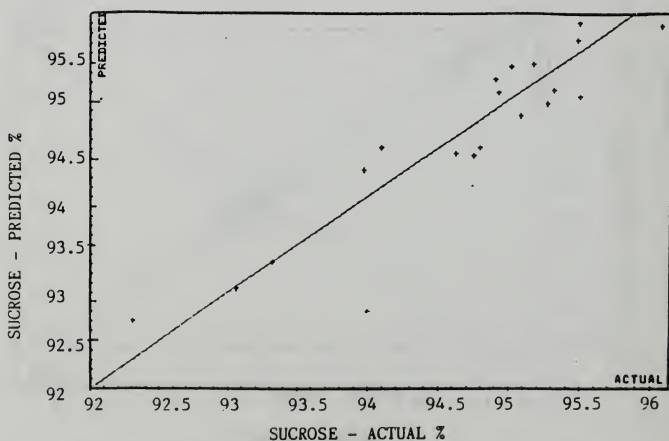


Figure 6. NIR correlation of sucrose in soft brown sugar

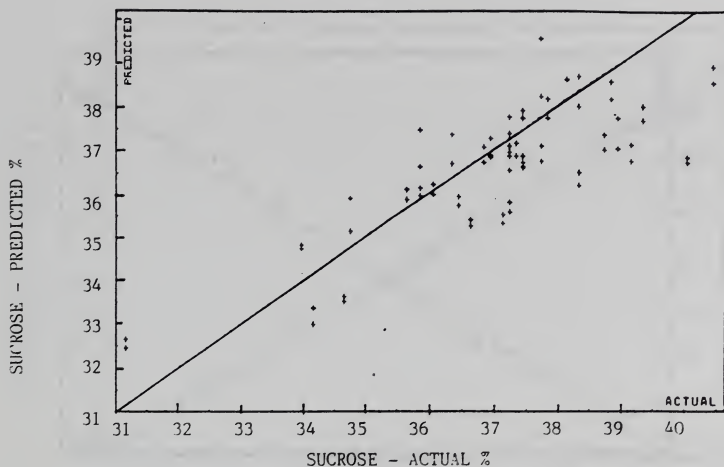


figure 7. Prediction of sucrose in Invicta C molasses by NIRA

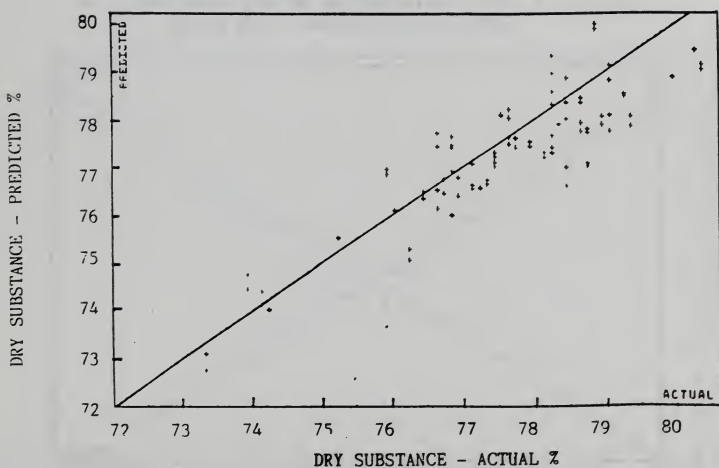


Figure 8. Prediction of dry substance in Invicta C molasses by NIRA

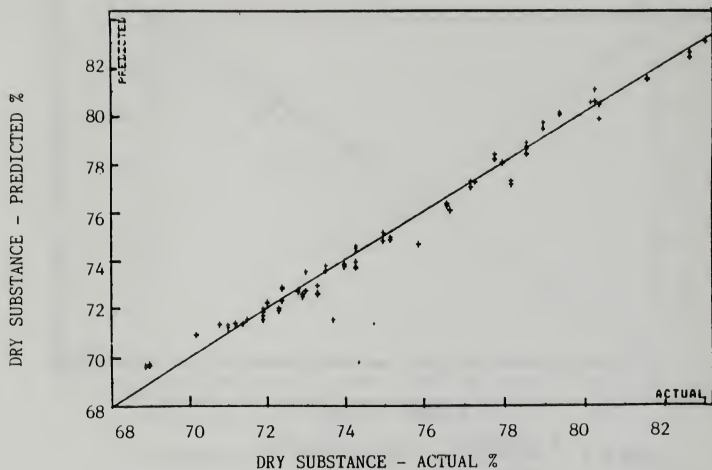


Figure 9. NIR correlation of dry substance in undiluted refinery BBO syrup

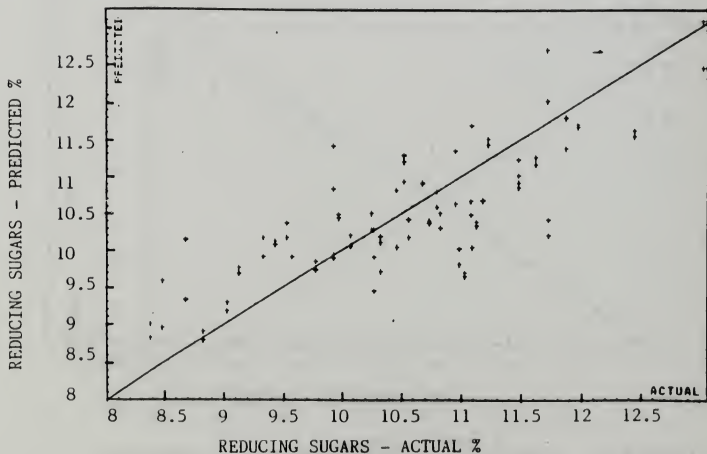


Figure 10. Correlation for reducing sugars in undiluted refinery BBO syrup

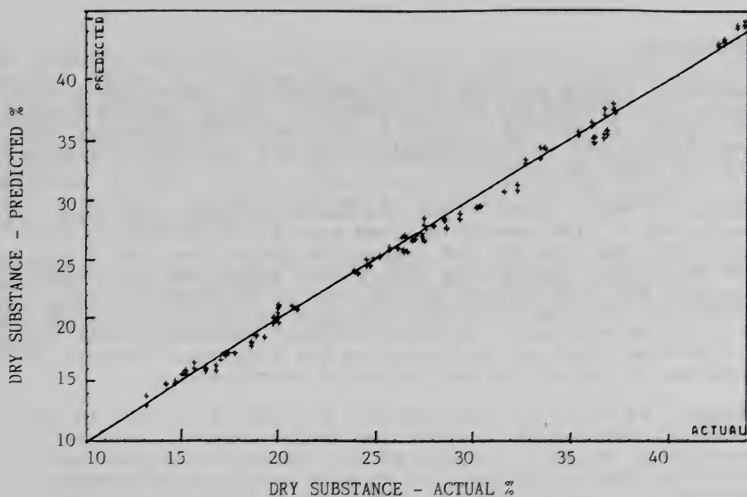


Figure 11. Prediction of dry substance in molasses broth by NIRA

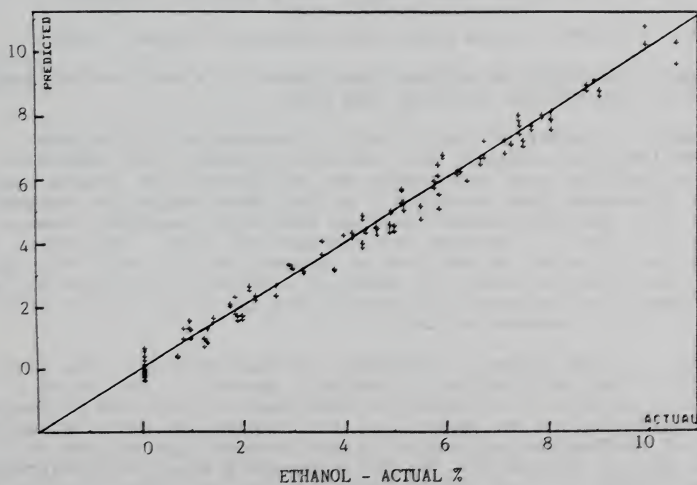


Figure 12. Prediction of ethanol content in molasses broth by NIRA

DISCUSSION

Question: I have considered this method for use on grain size, crystal elongation and other parameters. Since the grain size and crystal elongation are not independent from color and the other impurities in the crystals, what are you really looking at with the near infrared?

Player: I don't think I know the answer to that. We were fascinated to find when we grinded sugars and calibrated for grain size that the NIR did respond to grain size, but for a long time we thought that all we were really measuring was water molecules and that everything was somehow related to water molecules. Obviously when you are talking about grain size, that could not be the case. Perhaps Professor Mantovani might be able to shed some light on that question but like Prof. Vaccari said, sometimes it is curious how you get a correlation.

Comment: It is my opinion that the problem is related to the amount of light which is reflected by the sample. In this measurement, we have a matrix effect because we measure the reflected light. Obviously, if we have an elongated crystal, compared to a normal crystal, there are different faces and different color, we have a different reflected light so we have a different response and different results. If you take into account the matrix effect which influences the reflection of the light, we can obtain a correlation depending on the amount of light that is absorbed specifically by a certain compound.

Player: I think I agree with what Professor Vaccari said.

Comment: Sometimes we suppose that there is a very active midget inside the apparatus, working very hard.

Question: I wonder if there isn't a fundamental difference between the beet industry and the cane industry with regard to NIR, in that the beet represents the end product of growth cycle and is therefore less variable than the cane, which you harvest over a six- to eight-month campaign and which responds tremendously to rainfall, changes its composition, etc. Therefore, to try to set up a set of calibration curves for the cane crop is impossible because within 24 hours of a rainfall you have different composition in terms of reducing sugars, etc. I'd be interested in your comments of that.

Player: I think there is something in what you say. The variability in the calibration is terribly important. One point I failed to mention in my paper is that when we took the estimation of polarization in raw sugar into our factory, we found standard error of prediction was better in the factory than we had obtained in the laboratory. We got a standard error of prediction in the factory of 0.06, which isn't a whole lot worse than just

the ordinary laboratory variance of pol, whereas, as you saw in the lab, we had a variance of standard error of prediction of 0.107. I think what we were seeing in the factory was less variation in the samples we were trying to predict on, but they did say that it would not hold its prediction from one week to the next all that well. They would wander off, so maybe there is something in what you say: the inherent variability of the samples in the cane industry may be significantly different than that in the beet industry.

Comment: I might point out that there is variability in the beet industry; within 24 hours of freezing and thawing there is considerable difference in the non-sugars makeup of sugar beets. Fortunately, that doesn't happen all the time, but it does happen.

RAPID DETECTION OF YEAST IN LIQUID SUGAR PRODUCTS

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¹ Finnsugar Ltd.

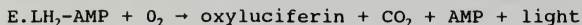
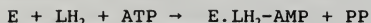
² Suomen Xyrofin Oy

³ Technical Research Centre of Finland (VTT)

INTRODUCTION

The detection of yeasts from liquid sugar and sugar syrups by conventional membrane filter techniques takes 3 to 5 days. In most cases the product is already used for its final application before the results are ready. The aim of this study was to find a rapid method which could be used in the quality control of sugar liquids. Two different methods - ATP-Bioluminescence and microcolony method were tested.

The firefly luminescence reaction has been the most extensively studied of the Bioluminescence reactions and is one of the most promising rapid microbiological tests tried to date. The firefly luminescence assay of ATP is based on the following reaction scheme (McElroy et al., 1969):



where E is luciferase, LH_2 is luciferin, $E.LH_2-AMP$ is enzyme bound luciferyl-adenylate, PP is pyrophosphate. When all reagents except ATP are in excess, the light in this reaction is proportional to the ATP concentration. Using standardised Bioluminescence reagents with a sensitive luminometer, ATP concentrations as low as $10^{-13}g$ can be detected. A linear relationship between ATP concentrations and the number of viable cells in a sample can also be established since cells contain relatively constant levels of ATP.

The assay of ATP Bioluminescence has been tested widely to food-stuffs and reagent kit applications e.g. for milk, fruit juices, beer and water are available (Lumac bv.). Very promising results have been obtained in carbonated beverages (LaRocco et al., 1985), in glucose syrups (Hamilton, 1989) and in the brewing industry (Simpson et al., 1989).

Microcolony method with optical brighteners as a rapid method of detecting low numbers of microbes in brewing industry has been reported (Haikara and Boije-Backman, 1982; Harrison and Webb, 1979; Parker, 1989). In this method samples are treated in the

same way as in the conventional membrane filter technique except adding optical brighteners into the growth medium. The incubation time is shorter and the membranes are examined under fluorescence microscopy. Optical brighteners are strongly fluorescent when excited by UV radiation. They are usually derivatives of diaminostilbenedisulphonic acid, distyryl- β -phenyl or pyrat-solsulfonic acid. Optical brighteners are taken up by mammalian cells and by wide range of micro-organisms by binding them to the proteins and chitin or to β -linked carbohydrates.

Another alternative is the staining of microcolonies after the incubation with fluorescent dye, e.g. with acridine orange. Acridine orange dye is bound to nucleic acids in microbial cells (Hobbie et al., 1977).

MATERIALS AND METHODS

Bioluminescence

Samples. Samples collected from the production line were first sterilised (121°C, 10 min.) and then spiked with Hansenula Anomala. H. anomala was previously isolated from liquid sugar. Twenty four hour culture, incubated in modified Wort agar at 30°C, was used to spike the liquid sugar samples. Also some quality control and syrup samples from the production were tested. Liquid sugar: d.s. 65%, colour 10 ICUMSA, sucrose > 99% of d.s. Syrup: d.s. 78%, colour 10000 ICUMSA, sucrose 35%, total sugars 94%.

Modified Wort agar. The yeast was cultured in Wort Broth Base (Merck Cat. No. 5449) with 300 g/l sucrose and 100 g/l dextrose. As a reference growth medium Wort agar (Difco No. 0111-17-7) with before mentioned amounts of sugars were used. Both were sterilised at 121°C, for 10 minutes.

Membranes. 0.8 μ m, 47 mm, sterilised cellulose-nitrate membranes were used (Sartorius SM 11404) for liquid sugar and 1.2 μ m, 47 mm positive charged membranes (Ultipor N66 NNGO 47100, Pall Process Filtration Ltd) for syrups.

Filtration. Vacuum filtration equipment (Millipore) with three filtration units were used.

Equipment and reagents. Lumac Biocounter M 2500, Lumac Beer Microbial Kit Cat. No. 9287-7, available from Lumac bv, Netherlands.

Procedure. 100 g or 200 g d.s. liquid sugar was diluted 1:1 with sterile 0.9% NaCl solution, vacuum-filtered, washed with NaCl solution, removed to Petri dish (50 mm), resuscitated for 1 h or

5 h and then treated according to the instruction from Beer Microbial Kit. Conventional membrane filter technique was used as a reference method. Plates were incubated for 72 h at 30°C.

Microcolony Method with Fluorescence Microscopy

Samples. As in Bioluminescence excluding quality control samples. In addition to H. anomala, Pichia guilliermondii (VTT-C-72064) was used in spiking.

Microscope. Polyvar Reichert Jung, HBO 200 W / 4-L1 with 10x eye-piece, 12.5x and 40x objective lens were used. Reichert module U1 for optical brighteners and B1 for acridine orange were used.

Membranes. Sterilised 0.45 µm, black cellulose-nitrate, 47 mm (Sartorius 13006) for optical brighteners and sterilised 0.6 µm, black polycarbonate, 25 mm (Nucleopore SN 100658) for acridine orange method.

Optical brighteners. Brighteners sterilised by filtering were added to sterilised modified Wort agar still in liquid form at the temperature at 50°C. Brighteners and the concentrations used are listed in table 1.

Table 1.--Optical brighteners and the concentrations used.

Optical brightener trade name*	Concentration /mg/l
UVITEX 2B 200%	100, 250, 500
UVITEX 2BT	100, 250, 500
Combination	
UVITEX 2B 200% : UVITEX CF 200%	100:300

* Producer: Ciba-Geigy

Optical brighteners procedure. 1 g or 10 g d.s. liquid sugar was filtered, membrane washed with NaCl solution, removed to Petri dishes with optical brighteners listed above. Plates were incubated at 30°C for 8 h, 14 h and 48 h. The membranes were examined under fluorescence microscopy. Only the last brightener in table 1 was used in testing syrups. The incubation times for syrups were 14 h, 24 h and 48 h. The reference method was the same as in Bioluminescence.

Acridine orange procedure. 1 g or 2.5 g d.s. liquid sugar was filtered. The plates were incubated for 8 h, 14 h, 24 h and 48 h at 30°C. The membrane was removed from the plate to the vacuum filter unit, treated with 2.5 ml 0.025% acridine orange for 2 min., washed with 2.5 ml 0.1M sterile filtered, pH 6.6 citrate buffer and 2.5 ml isopropanol, dry membrane was removed to slide and examined under fluorescence microscopy.

RESULTS

Bioluminescence

The quality control samples from the liquid sugar production were practically free of microbes. The results are shown in fig. 1 200 g d.s. of sugar liquid was filtered and it gave background ATP-level varying from 11 RLU to 16 RLU.

It is known that the ATP-values temporarily decrease just after the filtration. In order to test the resuscitation time needed the sterilised samples were spiked with Hansenula anomala cells. The results in fig. 2 show that one hour resuscitation after filtration is sufficient. Five hours incubation resulted in an increase in cell numbers.

The results in fig. 3 show how the ATP-value increases when the number of cells increases. It can also be seen that the differences between ATP-values are quite small with low cell numbers. According to our results at least 600 yeast cells per sample are needed to give detectable ATP-values. It means that when 200 g d.s. liquid sugar sample is filtered the lowest detectable yeast count is 30 cells per 10 g d.s.

Linear regression between the conventional membrane method and the bioluminescence method is shown in fig. 4. According to these results ATP-values correlated well with the cell counts achieved by the conventional method.

The results with brown sugar syrups were not successful. The filterability of brown syrup was much poorer than that of liquid sugar and only 10 g d.s. could be filtered by positive charged membranes. With normal cellulose-nitrate membranes almost nothing could be filtered. The ATP-values obtained could not be differentiated from the reference. Brown syrup analysis requires further studies.

Microcolony method with fluorescence microscopy

Microcolony method with optical brighteners was tested with Hansenula anomala and Pichia quilliermondii inoculated liquid sugar. The results with Hansenula anomala and Uvitex 2B 200% dye, concentration 250 mg/l, are shown in fig. 5. It can be seen that

after 14 h incubation cells with the brightener could be detected. After 24 hours the maximal cell count was achieved with optical brightener but no colonies were detected with the conventional method. The conventional method required 72 h to get the final cell count.

The results with P. guillermondii and Uvitex 2 BT dye, concentration 500 mg/l, were not as favourable compared to the conventional method (fig. 6). The colonies with optical brightener could be detected after 14 h but the cell count was remarkably lower than the cell count achieved with the conventional method after 24 hours incubation.

F-distribution was used to find out if the different concentrations of optical brighteners and different yeasts did have significant statistical difference compared to the conventional method.

After eight hours incubation growth could not be detected. After 14 hours incubation time there was no significant statistical difference compared to the reference method. $F_{12,4}$ for UVITEX 2BT was 1.00 and for UVITEX 2B 200% 0.71. For the mixture of UNITEX 2B 200% : UVITEX CF 200% $F_{6,2}$ value was 2.45. The higher was the concentration of optical brightener the easier was the detection of the colonies. No toxic effect could be detected at the concentrations of 500 mg/l of optical brighteners. UVITEX 2B 200% gave the weakest fluorescent.

With acridine orange staining reliable results were obtained also in 14 hours ($F_{6,2}=2.13$). The fluorescent was most intensive with acridine orange procedure.

Syrup. Two sided T-test was used to find out if there were any significant statistical differences between the microcolony method and the conventional method.

After 14 hours incubation growth could not be detected. After 24 hours incubation when the CFU level was $> 50/g$ d.s. no significant differences could be detected ($t_{0.05,4}=2.55$). When the CFU level was $< 50/g$ d.s. growth could not be detected.

CONCLUSIONS

With microcolony method reliable results were obtained after 14 hours for liquid sugar and after 24 hours for syrups at the level > 50 CFU/g d.s. Although the detection time was shorter than in the conventional method there is one major disadvantage: the method is far too labour-demanding for routine quality control. Bio-Foss Co. in England has developed (in 1989) an application where automated detection and enumeration of microcolonies using

optical brighteners and image analysis are used and very promising results are obtained in brewing industry (Parker, 1989). In this method plate reading and counting is quick - 20 plates/minute.

With the firefly bioluminescence method the results could be obtained in two hours from sampling. The detection level of yeast was 30 CFU per 10 g d.s. in liquid sugar when 200 g d.s. sample was filtered. In brown syrups our results were not very successful and further studies are needed. The specification limit (18 CFU/10 g d.s.) could not be accurately achieved in liquid sugar, but when the contamination problems arise the cell counts are very often higher than the detection level. That is why the bioluminescence-ATP-method seems to be a promising method and could be successfully used in the quality control of liquid sugars.

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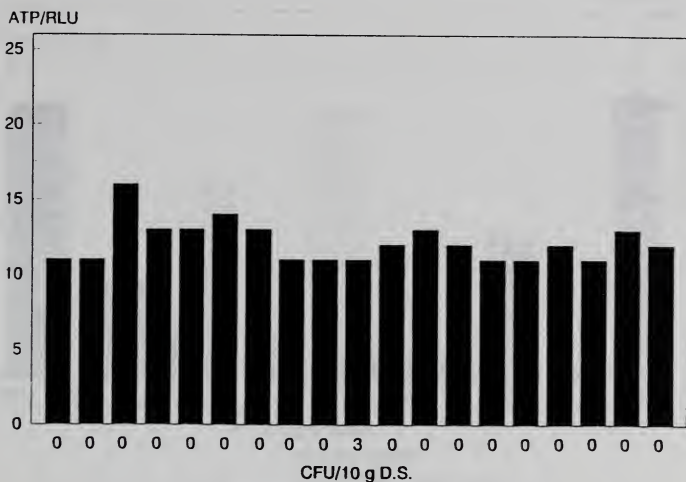


Figure 1.--ATP/RLU-values compared to CFU in liquid sugar quality control samples.

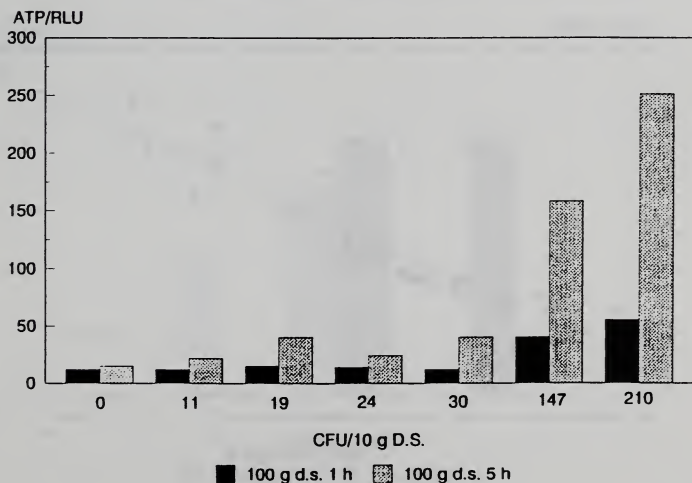


Figure 2.--The effect of resuscitation time on ATP/RLU-value in Hansenula anomala spiked liquid sugar.

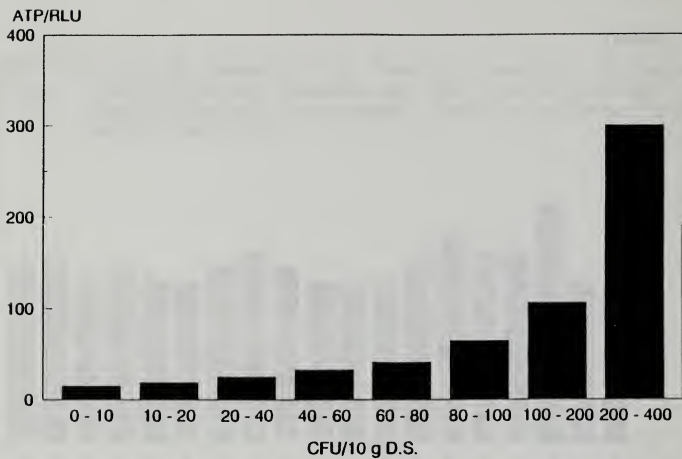


Figure 3.--ATP/RLU-values compared to CFU in Hansenula anomala spiked liquid sugar.

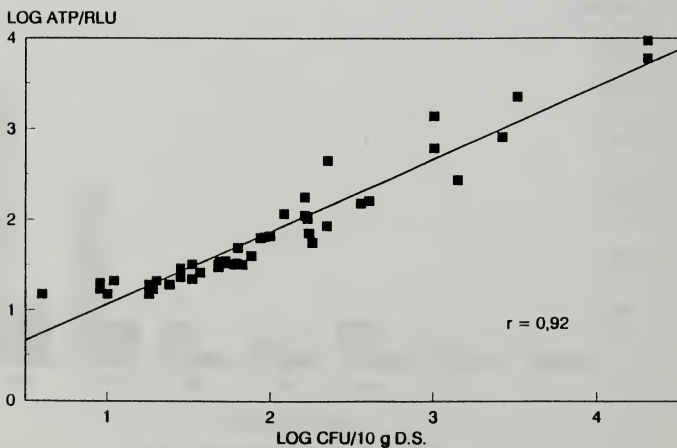


Figure 4.--Linear relationship between ATP/RLU and CPU in Hansenula anomala spiked liquid sugar.

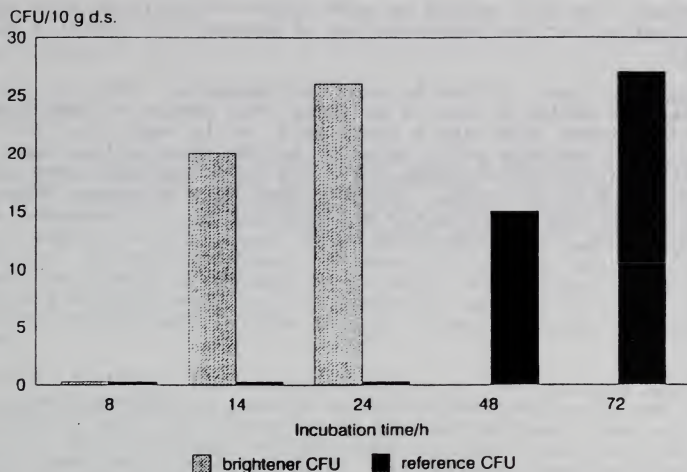


Figure 5.--CFU with optical brightener Uvitex 2 B 200% compared to conventional CFU in Hansenula anomala spiked liquid sugar.

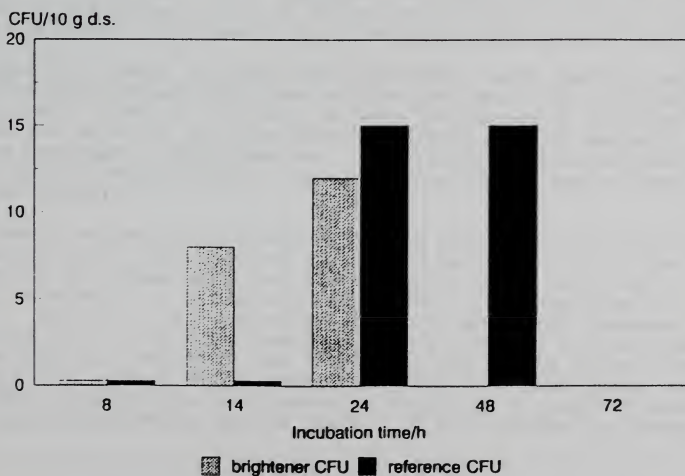


Figure 6.--CFU with optical brightener Uvitex 2 BT compared to conventional CFU in Pichia guilliermondii spiked liquid sugar.

DISCUSSION

Question: Can this method be used either directly or with some modification for the determination of mesophilic bacteria and molds?

Ahvenainen: Yes. It can be used for bacteria. The only problem is that the bacteria cell is so small, the amount of ATP is about 100 times lower than with a yeast cell, so it means that we have to have 100 bacteria cells compared to one yeast cell. Our detection level was 30 yeast cells, so it means 3,000 bacteria cells, which is too much, I think. That is a problem. This method is not specific to the microbial cells. It measures total ATP no matter where that comes from.

DEXTRAN IN RAW SUGAR

Thomas E. Wilson, Jr.

Colonial Sugars, Inc.

INTRODUCTION

There has been extensive documentation on the subject of dextran in cane juice and raw sugar and the resultant problems due to excessive levels in either juice or raw sugar. There have been several methods of analysis proposed to determine the levels of dextran and there are several methods currently in use throughout the world. At Colonial Sugars, we employ three (3) methods to analyze the levels of dextran in raw sugar and process liquors. The object of this paper is to discuss the use and reliability of the three methods and to offer Colonial Sugars opinion of these methods.

Dextran is formed by the bacterial action of the bacteria Leuconostoc mesenteroides on sucrose molecules and results in the formation of long chain, very gummy polysaccharides. The net effects of dextran formation are problems within the mill followed by problems for the refiner. The problems associated with excessive dextran levels include filtration problems leading to reduction in capacity and crystal distortion leading to elevated syrup purities. These are problems that can be avoided through proper cutting and milling practices, proper mill sanitation, and proper refinery operations. However; when there is a problem and dextrans are formed, it is important to have a reliable analysis method to determine the level of dextran in order to take appropriate measures and prevent the known problems that will occur.

At Colonial Sugars, we began our experiences with dextran methods with the Haze method which was written into raw sugar contracts in the early 1980's. In 1986, we were introduced to the Roberts method and began to analyze offshore cargoes by both methods due to the problems I will describe. And presently, we also use the SPRI Rapid Screening Test to check various sugars and process streams quickly.

The importance of the reliability of the method did not materialize to us until 1986. It was during the month of April that we found out the "hard way" that the Haze method did not give a complete picture of the levels of dextran in our incoming raw sugar. In April, we received an offshore cargo that appeared to be quite good. The polarization was 98.34 and the dextran was 0 MAU by the Haze method. This sugar was used as the majority of our melt for a week and we immediately began to experience severe problems in filtration and reduction in melt. In puzzled both our operational and technical staff. It was decided to contact

Sugar Processing Research to enlist their expertise in solving this problem.

RAW SUGAR ANALYSIS

Samples of the raw sugar and process liquors were sent to SPRI for analysis and the results indicated the raw sugar had a dextran level of 643 ppm as determined by the Roberts method. This explained the filtration problems we have experienced and indicted to us that there should be analysis by both the Haze method and the Roberts method for future cargoes. This also indicated that the Haze method was not always reliable in prediction of potential refining problems due to dextran levels. It is important that the method used be reliable to allow us to predict potential problems and prevent situations such as this from occurring.

The next problem we experienced with the reliability of the Haze method in prediction of refining problems was in 1987. During September of 1987, we received offshore raw sugar and performed the standard battery of tests. The sugar appeared to be acceptable and it was used as a substantial portion of the daily melt. The results of analysis by the Haze method indicated dextran levels in the range of 350-400 MAU. This is a moderate level of dextran and there was not a depression of daily melt as experienced previously. However; shortly after this sugar was used, our molasses purity began to rise and we could find no problems with techniques or equipment. The entire recovery side was checked and rechecked since we normally run at or below the "Theoretical Molasses Purity" as calculated based on the ratio of invert to ash in the raw sugar. Once again both our operational and technical staff were puzzled. We decided to analyze these domestic raw sugars and found dextran between 950 and 1090 ppm. Typically, we have learned that 300 - 400 ppm dextran will cause problems in the refinery. We also decided to check the crystals in our final strikes for elongation and we found elongated crystals and "needle-grain." This explained the elevated purity of the molasses since this "needle-grain" would pass through the centrifugal screens and into the molasses. Once again the Haze method alone did not allow us to predict that there would be a problem with the raw sugar.

In 1988, we had another problem with the reliability of the Haze method in prediction of potential problems. This problem occurred during the months of May, June, and July and was once again a case of melt loss due to filtration problems. In this situation, we had received several cargoes of domestic raw sugars and had stored these cargoes in such a way as to "block" the remainder of our raw sugar. We were forced to use these particular cargoes exclusively and the results were a depression in the

daily melt caused by severe filtration problems which we described as "char house restriction." The analysis of this raw sugar indicated less than 25 MAU dextran by the Haze method and between 211 ppm and 703 ppm by the Roberts method. The overall result in this case was a depression of the daily melt by a average of 13 percent with a maximum depression of 44 per cent.

1988 RAW SUGARS

Sugar (Origin)	Haze (MAU)	Problem Prediction	Roberts (ppm)	Problem Prediction	Actual Result
Domestic	9	OK	402	Problem	Problem
Domestic	9	OK	703	Problem	Problem
Domestic	24	OK	471	Problem	Problem
Domestic	23	OK	211	Marginal	Problem
Offshore	47	OK	41	OK	OK

Based upon these experiences, it has become standard practice at Colonial Sugars to analyze all offshore cargoes by both the Haze method and the Roberts method. The Haze is used to determine payment settlement since it is the method designated in the raw sugar contract; however, it is the Roberts method that is used to indicate the effect of the dextran within the raw sugar on the refinery. It is our opinion that the Roberts gives us a more complete picture of the dextran within the raw sugar; it allows us to predict the potential for problems associated with high levels of dextran in the raw sugar; and it allows us to use the raw sugars in a manner that prevents situations as previously described.

Unfortunately, both the Haze method and the Roberts method require several hours to perform which is usually no problem for the refiner with adequate storage and segregation capacity; however, most mills do not have this situation on the incoming cane. This is where the SPRI Rapid Screening Test for dextran can be used. This test is quick and will indicate presence or absence of dextran in the core sample juice. (We have used it on process liquor streams in the refinery with good results as well.) Armed with this knowledge, certain loads of cane or even loads of raw sugar can be segregated and processed according to their special needs.

SPRI RAPID SCREENING TEST

The SPRI Rapid Screening Test for dextran was employed during the 1988 Louisiana Raw Sugar Crop and the results point out that this test is an excellent indicator of incoming dextran in the core

juice. The graphs generated are based upon actual data for the 1988 grind and the core juice analysis is the SPRI Rapid Screening with the sugar analysis done by the Haze method. As the Graphs indicate, when there is an increase in the core juice dextran, there is a corresponding increase in the raw sugar dextran. Also, since the bacteria can enter the mill in the soil carried in with the loads, the graphs indicate that the higher the level of mud in cane the more likely the formation of dextran will occur. This test is a valuable tool for sugar mills to use to predict the level of dextrans entering their mill in the cane and then relaying this information back to the farmers. This offers the cane sugar industry a mechanism by which dextran can be measured and controlled at the beginning of the sugar processing cycle where it logically should be controlled.

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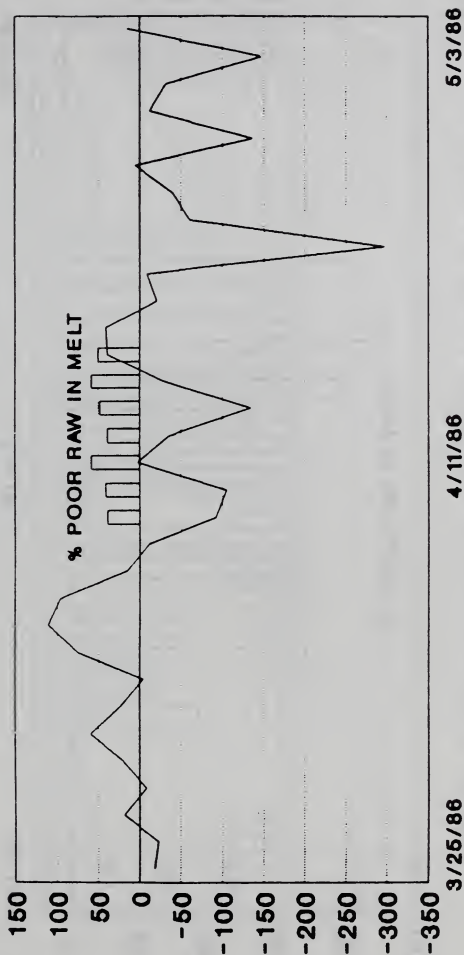
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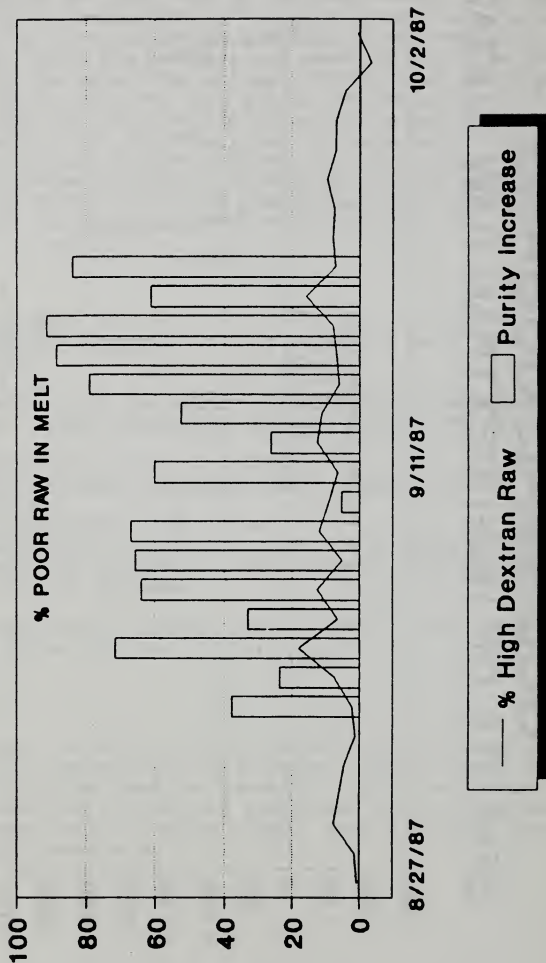
COLONIAL SUGARS

EFFECTS OF HIGH DEXTRAN RAW SUGAR



COLONIAL SUGARS

EFFECTS OF HIGH DEXTRAN RAW SUGAR ON INCREASE IN MOLASSES PURITY

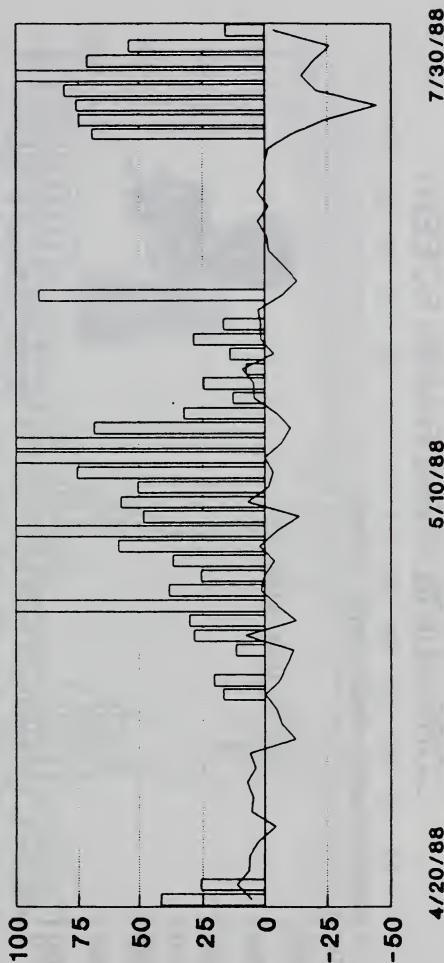


% INCREASE OVER THEORETICAL PURITY

COLONIAL SUGARS

EFFECTS OF HIGH DEXTRAN

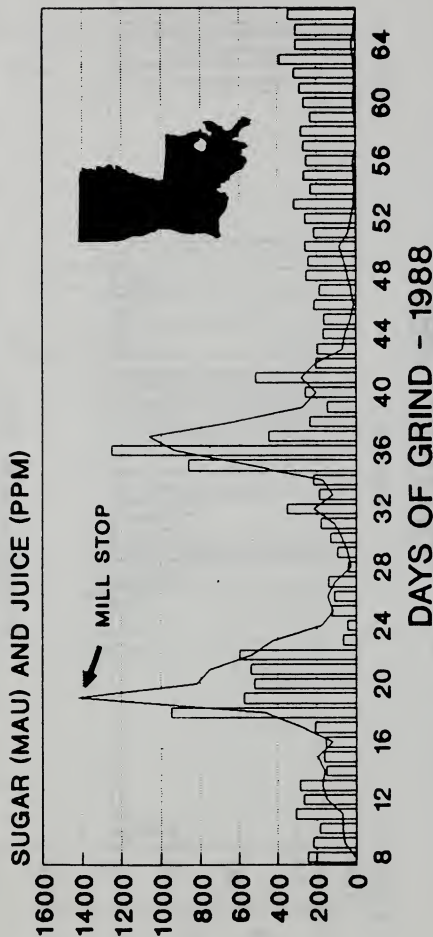
ON DAILY MELT



MELT IN TONS ABOVE/(BELOW) STANDARD

COLONIAL SUGARS

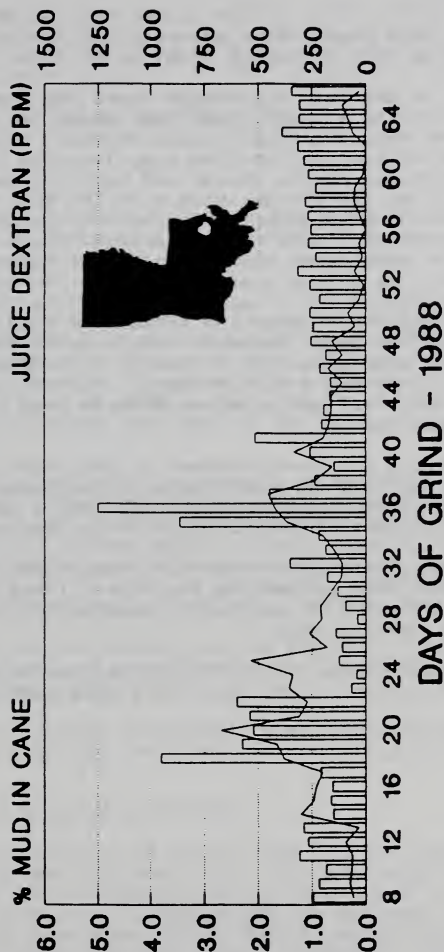
CORRELATION SUGAR (HAZE) AND CORE JUICE (SPRI RAPID SCREEN)



— Raw Sugar Dextran □ Core Juice Dextran

COLONIAL SUGARS

CORRELATION PERCENT MUD IN CANE AND CORE JUICE (SPRI RAPID SCREEN)



— % Mud In Cane Core Juice Dextran

DISCUSSION

Comment: This paper shows an excellent application of methods developed by SPRI to detect problems up front for the refinery.

Comment: In the last six months there has been work going on in Australia trying to understand the reason for the differences between the haze and Roberts test results. What was done was to include an enzyme confirmation step into the Roberts procedure, by splitting the sample stream and carrying the Roberts procedure on one-half of the stream, then treating the other half with a dextranase and then carrying the Roberts procedure on that stream as well. You then take the difference between the two results to give you an indication of dextran in the Roberts test precipitate. When the enzyme confirmation test is included in the Roberts procedure you generally get results that agree with the Haze test results. Those two tests were also compared with the new test by the Sugar Research Institute in Mackay based on an enzyme-HPLC procedure. It is really a reference test which takes two days, it is not a routine test. It gave comparable results with the haze test and with the Roberts test with an enzyme confirmation test.

I'm not saying you have problems in your factory, but your work indicates you have difficulties with performance of raw sugars which have a high Roberts test result and a low haze test, then due to non-dextran material in those raw sugars.

You suggested that with sugar no. 1 you experienced gel on your char filters when processing that raw. I believe that's about a certain indication of microbial infection in your process and not related to the raw sugar.

In the second cargo, my experience is that no one experiences problems in process with that low a haze test result.

SUGAR LIQUORS PURIFICATION USING ANIONIC RESINS WITH REGENERANT RECOVERY

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INTRODUCTION

Ion exchange resins have been used in the sugar industry for different purposes: softening, demineralization, decolourization, inversion, ion-exclusion and to change ions with different melassigenic effects.

Although the resins have proved to be a good tool in the sugar industry, some problems arise with their usage. Waste disposal is the main problem involving resin applications. In the liquor decolourization 6 to 9 m3 of regeneration coloured effluent, are produced per 100 tons of white sugar refined. In the sugar industry, these effluents are some of the most difficult to treat (Glabsky, 1986).

A technique to recirculate a great part of the salt effluent was developed recently, involving the separation of effluent colourants according to their affinity to the resin and precipitating with lime those with higher anionic charge (Bento, 1989). The treated effluent is re-used as regenerant and the alkaline diluted solutions can be used to regenerate weak base anionic resins used as protection for the main decolourization resin.

Further studies and development of the effluent treatment, resulted in a process of simultaneous decolourization and decalcification of sugar solutions.

The description of these techniques, when using carbonated liquors from a cane sugar refinery, is the object of this paper.

TREATMENT OF THE REGENERANT EFFLUENT

During the regeneration of a strong base anionic resin, used as cane sugar liquor decolourizer, the colourants are removed from the resin as the NaCl concentration increases and according to their affinity (Fig. 1). By observing the Effluent Attenuation Curve we see that more than 50% of the colourants are desorbed with less than 10% of the total salt consumed in the regeneration.

In the beginning of the regeneration process the resin is surrounded by water from the last resin wash. As the salt solution penetrates the resin column the chloride concentration increases

and the colourants are displaced from the resin. The colourants of low affinity are displaced at a low salt concentration and those with a higher charge are eluted more slowly (Williams, 1988) and are displaced only at higher salt concentrations. These two kinds of colourants can be better separated by making a pre-regeneration at a low salt concentration before the main regeneration (Fig. 2). In this curve we indicate the LV of the different fractions (Fig. 2). In this curve we indicate the LV of the different fractions (LV = lime value - related to the decolourization obtained when the effluent salt solution is treated with lime under standard conditions (Materials and Methods) - the LV of the effluent fractions depends on the colourants anionic charge).

Treating with lime the last part of the regeneration effluent (with high LV), the anionic colourants are precipitated and the solution, after filtration, can be recirculated and used as regenerant (Fig. 3). The colourants that do not precipitate with lime and remain in solution after the treatment do not interfere with the regeneration process, as their anionic charge is not sufficient enough to compete with the chloride ions.

A quantity of 5 to 10% (v/v) of calcium hydroxide slurry containing 100 g/l of $\text{Ca}(\text{OH})_2$ is used, in a quantity depending on the colourants contained in the effluent. The excess of calcium in solution is precipitated by sodium carbonate or other calcium precipitating agents, such as trisodium phosphate or carbon dioxide.

The ion exchange process with regenerant recovery was tried in a Lab scale and in a Pilot Plant. One column with 100 ml of resin IRA 900 C was used in the lab experiments. After each effluent treatment the filtrate solution was used as regenerant in the next cycle. A salt make-up, amounting to 44 g/liter of resin/cycle, was done to replace the salt losses. The experiment was done for 200 liquor cycles. Every 20 cycles a special acid-/alkaline regeneration was done using fresh salt. The total salt used per cycle, including the special regenerations, was 77 g/liter of resin.

During the effluent treatment the average consumption of chemicals was 22 g of $\text{Ca}(\text{OH})_2$ and 16 g of Na_2CO_3 per liter of resin per cycle (when sodium carbonate was used). This consumption represents a reduction of almost 50% in chemical costs, when compared with classic regeneration system (at local prices).

The only liquid waste of the process contains the colourants from the first part of the regeneration that are discharged as a solution with a low salt concentration. This waste amounts to 1.0 liter per liter of resin per cycle and has, on average, a salt concentration of 23 g/l of NaCl , an Atten. (420 nm) of 8600 and a

pH of 9.6. A process to treat this effluent in order to increase the colourants LV allowing their removal by lime precipitation is under progress at RAR. In this process the colourants in the rejected effluent are oxidised and precipitated with lime. The pollution charge of the rejected effluent is then reduced.

The resin behaviour, after regeneration with the recovered salt, was normal, as observed in Table I. After 200 cycles the resin presented a strong-base capacity of 0.58 meq/l.

This process was also tried in a two resins system using a weak base anionic resin before the strong base resin.

It is known that weak basic resins remove efficiently large organic molecules and other colourants such as Caramels and ADPs (Raden and Anderson, 1970). The process described earlier was adapted, in a Lab scale, to a two resins system with 100 ml of a weak base anionic resin (Lewatite MP62) and 100 ml of a strong base anionic resin (IRA 900 C) (Fig. 4). The weak base anionic resin was regenerated with the alkaline effluent from the final part of the strong base resin regeneration. A quantity of 3.5 g of NaOH was used per liter of resin per cycle to maintain the effluent at a pH of 13.

The feed liquor colour and the decolourization obtained during 200 cycles are presented in Table II and Fig. 5. The analysis of the effluent treatment are presented in Table III. For the effluent treatment the quantity of chemicals used was identical to that used for the experiment where one resin was used. The NaCl make-up was 41 g per liter of resin per cycle and the liquid waste was of 2.0 liters per liter of resin per cycle, containing 15 g/l of NaCl.

The average results in comparison with those of the system using only a strong base resin are presented in Table I. It is observed that the weak base resin gave some protection to the strong base resin which after 200 cycles presented a strong base capacity of 0.80 meq/liter. In the first part of the experiment the weak base resin contributed to 50% of colour removal. This value dropped to 19% in the last cycles.

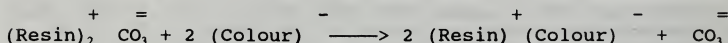
A pilot plant was installed at RAR Oporto Refinery in order to test the resin effluent treatment as described earlier. The initial batch installation was changed, after the first experiments, to a continuous flow system (Fig. 6). The final lay-out consisted of a system with two reactors with a capacity for treatment of one cubic meter of regenerant effluent per hour. In the first reactor the salt effluent is reacted with lime at a controlled temperature. By over-flow the solution flows to the second reactor where the sodium carbonate precipitates the excess calcium in solution. Dosing pumps make the necessary chemical dosing. The treated liquor is then filtered through a 2 m² plate

and frame polypropylene filter. The treated effluent was used, without any problem, directly in the refinery resin systems. Each filter cycle represents on average 3.4 m³ of the treated effluent with 71 g/l of NaCl. The decolourization obtained was 42% and the treated effluent had a Attenuation of 2300 and a pH of 12.9. On average the chemical consumptions with the pilot plant was 8.5% of Ca(OH)₂ and 5.8% of Na₂CO₃ (on solid salt). The costs of these chemicals represent 37% of the value of the recovered salt (at local prices).

DECOLOURIZATION/DECALCIFICATION OF SUGAR SOLUTIONS

When an excess of sodium carbonate was used to precipitate the calcium, during the salt effluent treatment described earlier, the sugar liquor became cloudy. A calcium carbonate precipitate was observed. At the beginning, the problem was overcome by controlling the sodium carbonate quantity and by adjusting the final solution pH. Later, it was considered that this inconvenience could be an advantage by using it as a decalcification process with simultaneous colour removal, for sugar solutions.

A process was developed consisting of changing the resin counter ion from the chloride to the carbonate ion. This was done by passing a sodium carbonate solution through the resin, after the sodium chloride regeneration. The carbonate ions bound to the fixed ions of the resin are exchanged by the anionic sugar colourants and released to the solution. When in solution, the carbonate ions react with the calcium ions and the calcium carbonate precipitates.



In this process the calcium ions are removed without adding an extra cation to the sugar solution. One part of the calcium carbonate (10%) is mixed with the decolourized liquor that must be filtered after the resins. It was observed that only the first 5 BV of treated liquor presented a cloudy appearance due to the calcium carbonate precipitate. The rest of the liquor effluent was clear.

Another part of the calcium carbonate (60%) remains fixed on the resins. This can be dangerous because caking of the resins may

occur if an excess of calcium carbonate is fixed to the resin. From our experience, in the Lab column of 1 liter of resin, serious caking rarely occurred and, even on those occasions where this did occur, the resin became free flowing after diluted acid (HCl) treatment. When a level of CaO under 500 ppm/DS and a total charge load under 20 g of CaO per liter of resin per cycle was maintained, resin caking never occurred.

This process can overcome one problem of resin utilisation, that is, their inability to remove ash (Sheahan, 1990). Calcium removal from carbonated liquors is important due to the possibility of calcium salt precipitation during evaporation and crystallization, or any possible turbidity in liquid sugars (Lancrenon, 1988). Also by removing calcium from liquors, an increase in their flavours are obtained, which is important for soft sugar production.

This process was tried in a one liter resin column, charged with IRA 900 C, with a water jacket at 60°C. The carbonate liquor from Oporto Refinery was passed at a flow of two liter per hour and in a quantity between 20 and 50 BV, depending on feed liquor colour and calcium content.

Resin regeneration was performed in various steps. In the first step the calcium carbonate deposited on the resin was removed by gassing the resin, in water, with carbon dioxide. Gas was injected into the resin through a distributor inside the resin support silex. Hot water was fed, in up flow, at a rate of 2.0 BV/h and in a quantity of 6.0 BV. Calcium removed in this operation was circa of 6 g per cycle. Effluent gassed water reached a pH of 5.8 on average. This operation was also performed in a batch way using 3.0 to 4.0 BV of water after one hour of bubbling gas. A separatory funnel was installed on the top of the resin column in order to separate the resin pushed out of the column by the gas flow. Gas was supplied by a CO₂ pressure container. When in the refinery, CO₂ can be obtained from the carbonatation gas compressors. An acid washing with diluted solution of hydrochloric acid was also used, with success, to remove the calcium carbonate.

In the second step a sodium chloride solution was used in order to remove the sugar colourants fixed to the resin.

In the third step a sodium carbonate solution was used in order to change the resin from the chloride to the carbonate form. Before this step a water washing can be used to avoid contamination of the soda solution with sugar colourants. As the sodium carbonate is used in excess, the clear soda solution out of the resin can be re-used in the next regeneration step, with a Na₂CO₃ make-up.

The decolourization with simultaneous decalcification process was tried during 40 cycles in which different regeneration processes were used. In Table IV are presented the results of these experiments. The decolourization and calcium leakage through one cycle (Colour in = 982; CaO in = 484 ppm/DS) is presented in Fig. 7 (Decolourization = 88.9%; Decalcification = 70.2%). We observed that up to 30 BV of liquor, CaO leakage was less than 50 ppm (93.8% of decolourization and 89.2% of CaO removal). The length of liquor cycle depends on calcium and colour of the feed liquor and on the limits chosen for these parameters in the liquor produced.

This process (Patent in process) is very convenient as we use only one resin type, an anionic resin, to remove an anion and a cation in the same operation, with good decolorization and decalcification. It seems that calcium carbonate precipitation does not affect the resin capacity as a decolourizer.

Another attempt to re-use the sodium carbonate effluent was to use it before the sodium chloride regeneration or mixed with the salt solution. It was observed that by doing so, a greater removal of colourants from the resins was obtained. In fact when using 1.5 BV of sodium chloride solution at 100 g/l mixed with 100 g/l of sodium carbonate, a greater amount of colourants were removed than when 3.0 BV of salt was used (considering Attenuation = Concentration of Colourants) (Fig. 8). Sodium carbonate is inefficient in the removal of sugar colourants from strong base polystyrenic resins (Fig. 9). As was observed, a mixed solution of sodium chloride and sodium carbonate has a great removal efficiency of sugar colourants from the resins. Chloride ions, at high concentrations, are water structure breakers (Fenamen, 1985) and will enter the resin phase with ease (Steigman, 1968). Other ions that are water structure formers enter the resin phase with difficulty (Steigman, 1968). Carbonate is one of these ions. When these two kind of ions are in the presence of the resin there will be an initial preferential penetration, into the resin phase, for the ion that breaks the water structure. The disorganised water inside the resin pores, due to the high concentration of the resin ions, counter ions and by the hydrocarbon matrix, referred by Diamond (Steigman, 1968), may explain this behaviour. This effect can provoke an induced increase of chloride activity in the resin phase that explains the high colourants removal when soda is mixed with the salt solution during regeneration.

The utilisation of a compound, such as Na_2CO_3 , in this case, to induce an activity increase of chloride ions inside the resin phase can only be of interest if the compound is cheaper than salt or with the possibility of being recovered or, as in our case, less pollutant than salt and is available.

The processes described, regenerant effluent treatment, simultaneous liquor decolourization and decalcification, and the regeneration with sodium chloride and sodium carbonate, can be used separately or simultaneously (Fig. 10). For the integral process of decolourization and decalcification of sugar solutions with regenerant recovery we anticipate a chemical consumption of circa 50 g of NaCl and 100 g of Na₂CO₃ per liter of resin per cycle, besides the chemicals used for the effluent treatment.

MATERIALS AND METHODS

Lime test. For coloured salt effluent from decolourization ion-exchange resins with an Attenuation (420 nm and pH 9) higher than 500.

200 ml of coloured effluent solution was taken and adjusted to pH 9 with NaOH or HCl. Attenuation of this solution was adjusted to 500 ± 50 by dilution with distilled water. Two 100 ml portions of this solution were taken and heated in a water bath to 60°C. To one of the portions (A) 3 ml of Calcium Hydroxide slurry with 100 g/l of Ca(OH)₂ was added. To the other portion (B) was added 3 ml of distilled water. Both solutions were heated for 5 minutes at 60°C with gentle agitation. The precipitate formed in portion (A) was filtered by vacuum through Whatman filter n°42. The pH of both solutions was adjusted, as previously, to pH 9. The Attenuation of both solutions was measured at 420 nm. Lime Value was calculated as:

$$L.V. = \frac{\text{Atten. (B)} - \text{Atten. (A)}}{\text{Atten. (B)}} \times 10$$

Attenuation. Measured with a spectrophotometer Perkin-Elmer LC-55 B with a 1 cm cell

$$\text{Attenuation} = \frac{\text{Absorbance} \times 1000}{\text{Cell length (cm)}}$$

Colour removal from resin. Charged resin (Lewatite MP500A) from the refinery column (with 32 cycles) was washed and charged in the Lab with 20 BV of carbonated liquor in batches of 2 BV for 2 hours with agitation at a temperature of 40°C. The charged resin was sweetened off, washed with distilled water and air

dried. In a closed flask, 10 g of this resin was contacted with 50 ml of different solutions (NaCl and Na₂CO₃ at 20, 40, 60, 80 and 100 g/l and a mixture of these compounds (20+20) g/l, ...) for 4 hours with agitation and at 40°C. Attenuation of the filtered solution was determined at pH 9.

Calcium determination. By EDTA and Eriochrome T.

Chlorine determination. By Mohr Method

ABBREVIATIONS

ADP - Alkaline Degradation Products
BV - Bed Volumes
CCU - Colour Charge Unity
DS - Dry Solids
H - Heating
HDE - High Density Effluent
HDR - High Density Regenerant
LDE - Low Density Effluent
LDR - Low Density Regenerant
LV - Lime Value
RE - Rejected Effluent
S - Sodium Carbonate
SB - Strongly Basic Resin
W - Water
WB - Weakly Basic Resin

CONCLUSIONS

Sugar refining involves successive steps of purity and impurity separations. Colour is one of the sugar impurities that provokes great worries. Anionic resins are a good instrument to remove colourants but they produce large quantities of salt effluents with a high charge of organic matter and a high salt concentration. By precipitating the effluents anionic colourants with lime and re-using the filtered solution, the waste disposal problem is reduced. Using a weak base anionic resin before the strong base resin increases the resin efficiency. Changing the counter ion of the resin from chloride to carbonate ion provokes a simultaneous removal of colourants and calcium ions from the liquor treated by the resin. Colourants are exchanged for the carbonate ions that, in turn, precipitate the calcium ions in solution. Calcium carbonate is removed from the resins with carbon dioxide and the colorants are removed by sodium chloride regeneration. The soda effluent can be re-used as soda solution to regenerate weak base resins used as protection to the strong base resins, or with the salt solution increasing the colourants removal from the resin.

These combined processes of resin usage in the sugar industry offer the possibility of reducing the chemical costs, increasing the liquor purity, reducing the effluent pollution, and a reduction in equipment to perform both liquor decolourization and decalcification in a single operation.

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Table I - Results of Lab Experiments.		One Column	Two Columns
Weak Base Anionic Resin			100 ml
Strong Base Anionic Resin		100 ml	100 ml
Number of cycles		200	200
Liquor In	BV/cycle	46	49
	Brix	63.2	63.6
	I.Colour	669	640
	C.C.U./cycle (*)	26.3	25.3
	pH	8.6	8.5
Liquor Out	I.Colour	180	120
	pH	7.7	8.7
Decolourization %		73.1	81.5
Resin Strong Base Capacity (after 200 cycles)		0.58 meq/l	0.80 meq/l

Table II - Liquor decolourization by ion-exchange with regenerant recovery

Cycles	Liquor In					Liquor Out		Dec. %
	BV	Brix	I.Colour	c.c.u.(*)	pH	I.Colour	pH	
1-10	50	64.5	513	21.8	9.0	66	9.2	87.1
11-20	50	64.4	570	24.1	8.6	86	8.9	84.9
21-30	50	62.4	587	23.8	8.5	81	8.8	86.2
31-40	52	64.0	524	22.3	8.6	46	8.7	91.2
41-50	50	63.6	621	25.7	8.6	80	8.8	87.1
51-60	46	63.3	704	26.8	8.2	88	8.6	87.5
61-70	49	63.6	690	27.8	8.4	90	8.6	87.0
71-80	49	61.4	600	23.2	8.4	86	8.6	85.7
81-90	46	60.5	699	25.0	7.7	107	8.4	84.7
91-100	46	63.9	744	28.0	9.4	156	9.4	79.0
101-110	50	63.9	702	29.2	8.8	128	8.6	81.8
111-120	49	62.2	723	28.6	8.0	124	8.4	82.8
121-130	50	62.9	602	24.6	8.4	104	8.4	82.7
131-140	50	63.8	562	23.6	8.8	106	8.4	81.1
141-150	48	61.9	760	25.4	8.6	137	8.2	82.0
151-160	51	64.3	518	22.1	8.4	124	8.7	76.1
161-170	46	65.3	732	27.8	8.0	186	8.4	74.6
171-180	46	65.3	618	22.8	8.8	183	8.8	70.4
181-190	50	64.4	564	23.6	8.8	173	8.8	69.3
191-200	46	65.4	772	29.6	8.6	240	8.6	68.9
Average	49	63.6	640	25.3	8.5	120	8.7	81.5

(*) - C.C.U.:colour charge unity - Tons of Dry Solids * I.Colour/litre of resin.

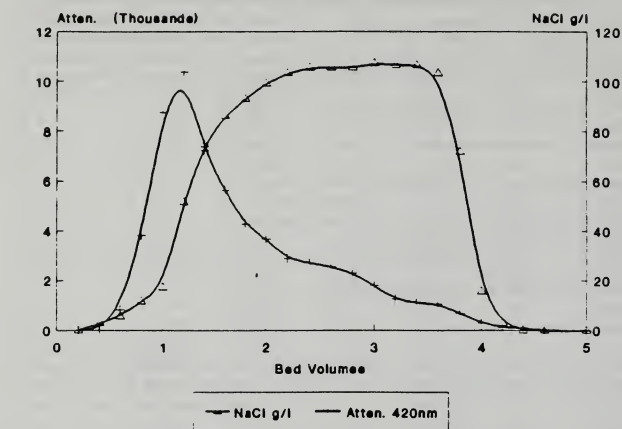
Table III - Coloured Effluent Treatment

Cycles	Before Treatment			After Treatment		NaCl Make-up g/l/cycle (**)
	NaCl g/l	atten. (*)	pH	atten. (*)	Decol. %	
1-10	80	4.78	12.2	2.81	41.2	62
11-20	76	9.90	12.2	7.67	22.5	50
21-30	72	7.06	12.2	5.68	19.5	38
31-40	72	10.26	12.6	9.00	12.3	60
41-50	64	10.28	12.9	8.97	12.7	38
51-60	70	13.70	13.2	12.72	7.2	50
61-70	72	10.14	12.4	8.77	13.5	36
71-80	68	14.32	13.0	12.68	11.5	52
81-90	85	6.80	10.2	5.04	25.9	58
91-100	78	11.71	11.4	9.16	21.8	68
101-110	76	11.06	10.0	9.12	17.5	24
111-120	70	7.88	9.8	6.45	18.1	36
121-130	74	9.34	10.0	8.02	14.1	25
131-140	72	9.30	10.3	8.11	12.8	28
141-150	79	12.63	10.1	9.34	26.0	34
151-160	74	9.36	10.0	7.13	23.8	30
161-170	78	10.64	9.8	7.20	32.3	36
171-180	73	10.07	10.4	7.66	23.9	24
181-190	74	10.02	10.4	8.08	19.4	36
191-200	73	11.86	10.2	9.32	21.4	37
Average	74	10.06	11.2	8.15	19.9	41

Table IV - Decolourization and Decalcification of Carbonated Liquor

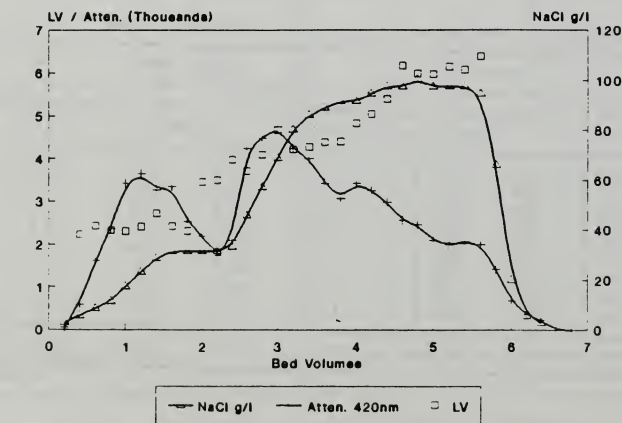
Serial # (5 cyc.)	Liquor In					Liquor Out		Colour Reduction %	Calcium Reduction %
	BV	Brix	Colour	CaO ppm/DS	CaO loaded g/l res/cyc	Colour	CaO ppm/DS		
1	50	63.9	569	278	11.5	77	41	86.5	85.3
2	50	63.2	658	251	10.4	94	62	85.7	75.3
3	50	64.6	760	351	14.9	168	152	77.9	56.7
4	50	65.6	748	479	20.7	207	294	72.3	38.6
5	44	65.2	941	413	15.6	161	146	82.9	64.6
6	28	64.9	1120	345	8.2	140	94	87.5	72.8
7	30	65.8	1059	425	11.1	178	127	83.2	70.1
8	32	65.2	981	401	11.0	168	105	82.9	73.8
Average	42	64.8	855	368	12.9	149	128	82.4	67.2

Fig.1 - Normal Regeneration
90 min - 110 g/l NaCl



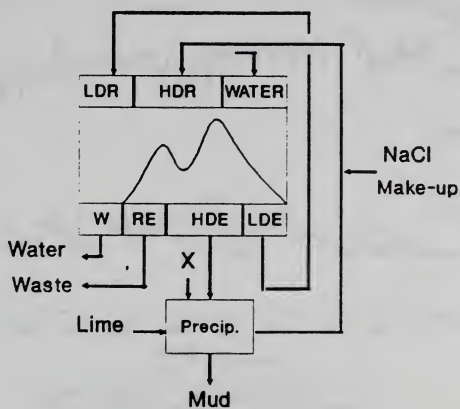
RAR-02

Fig.2 - Special Regeneration
60 min . 30 g/l + 90 min . 100 g/l NaCl



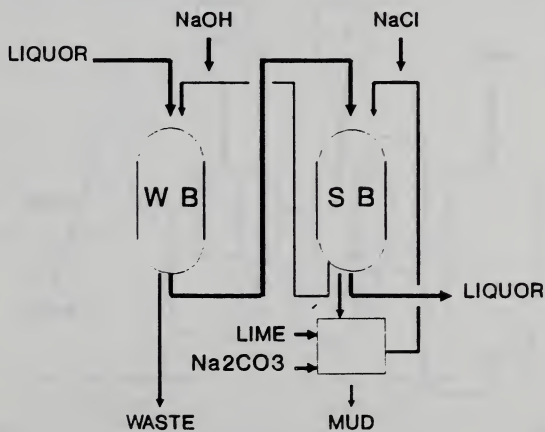
RAR-03

Fig.3 - Regeneration flow diagram.



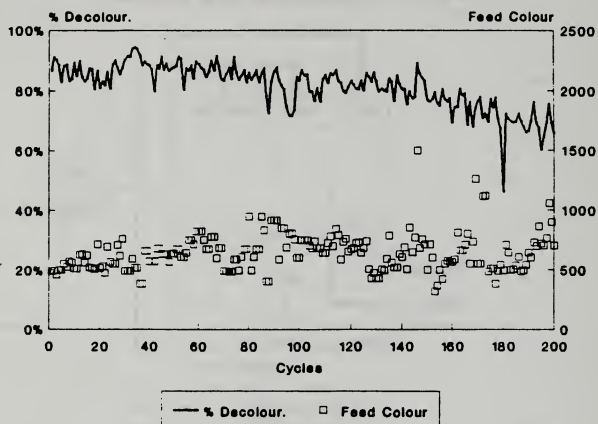
RAR-17

Fig.4 - Flow Diagram of Weak and Strong Base Resin



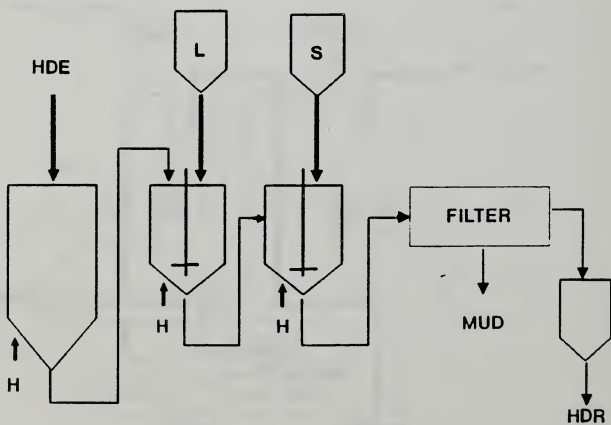
RAR-18

Fig.5 - Two Resins System
WB + SB



RAR-08

Fig.6 - Pilot Plant



RAR-28

Fig.7 - Decolourisation and CaO leakage

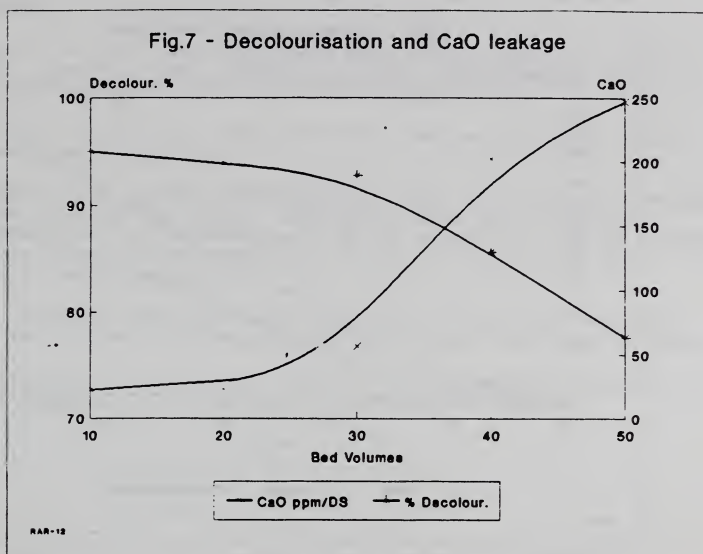


Fig. 8 - Regeneration with NaCl+Na₂CO₃

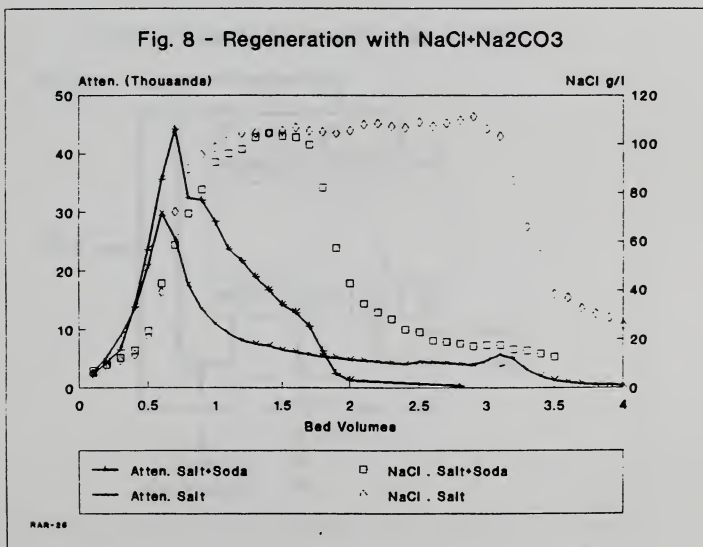
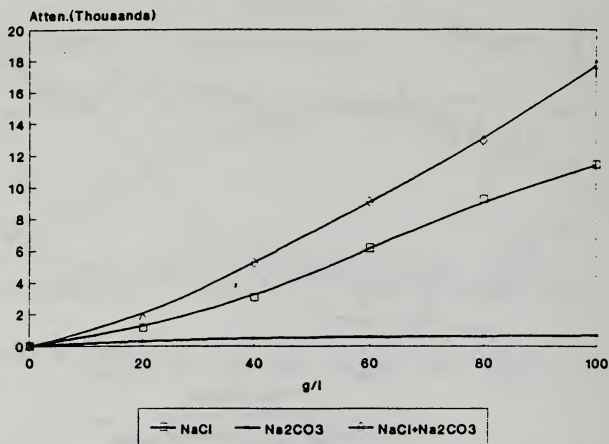
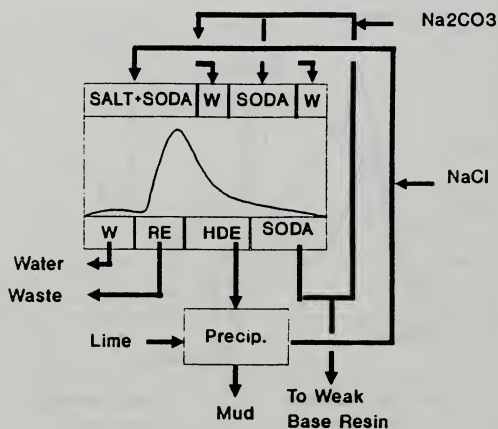


Fig.9 - Colour removal from resin



RAR-87

Fig. 10 - Flow Diagram of Integral Process



RAR-88

DISCUSSION

Question: In your work with weak base resin, you used styrenic resins. Have you also looked at acrylic weak base, or acrylic strong base preceding the styrenic resins?

Bento: No, we have not done that yet. We are planning trials with weak base acrylics.

Question: It's really interesting to see a new process developed right on site at the plant. Have you looked at the types of colorants that you're removing - or not removing. You're working with cane sugar in your refinery - have you also tried the process on beet sugars?

Bento: RAR receives cane sugar and that is our main interest, but we have tried the process with beet sugar with some success.

As to the kind of colorants - we are working on HPLC methods to look at those.

ULTRAFILTRATION: A NEW ALTERNATIVE FOR THE MANAGEMENT OF REGENERANT WASTE STREAMS

James R. Wilson and Robert W. Percival

Rohm and Haas Co.

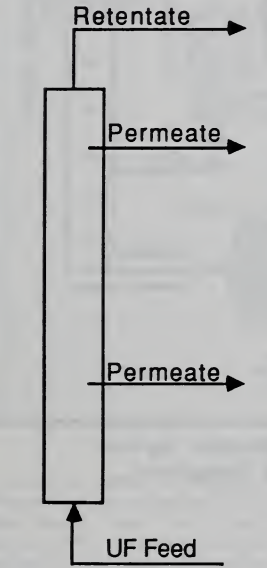
Worldwide concerns regarding industrial pollution and governmental regulatory sanctions are strong driving forces towards improving and developing waste management systems. The sugar refining industry is no exception to the problems associated with plant discharges. Hence, developing cost effective methods for achieving regulatory compliance has become necessary. Hollow fiber cross-flow ultrafiltration has been studied in the laboratory for the management of waste streams resulting from the regeneration of ion exchange materials employed to decolorize cane sugar.

Cane sugar decolorization involves many steps, including the contacting of the sugar syrup with strongly basic ion exchange resin which adsorb and exchange significant amounts of color bodies from the sugar. One of the advantages of using ion exchange resins to decolorize sugar is that the chemical reactions by which these organic molecules are removed from the sugar, and become associated with the resin, are reversible. Typically, the ion exchange materials are contacted with a 10% sodium chloride / 0.2% sodium hydroxide solution during regeneration. This regenerant is very effective in removing the color bodies from the resin; thereby, preparing the resin for reuse. The spent regenerant contains copious amounts of organic matter with 1,000 to 3,000 ppm of carbon being typical. Of course, these concentrations vary by refinery and are primarily a function of influent sugar color, service run length and regenerant dosage. The disposal of this spent regenerant represents one of the waste streams which sugar refineries find problematic.

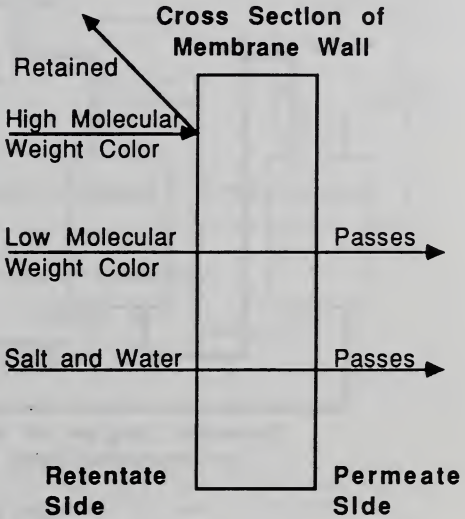
Processing waste bring regenerant by ultrafiltration (UF) can assist in the management of this material by effectively reducing the volume and organic concentration for disposal. This is achieved by passing the spent brine through an ultrafiltration system which retains molecular species larger than the size of the membrane pores, while permitting low molecular weight species, water, and salts to pass through the membrane. As the UF system continues to run, the concentration of retained organic matter increases and, conversely, its volume decreases. The volume of permeate, that solution which passes through the hollow fiber membrane wall, continues to increase as the UF system runs.

Romicon hollow fiber polysulfone ultrafiltration systems operate in a very unique fashion. The system operates upflow and permeation through the membrane wall is tangential to the flow of

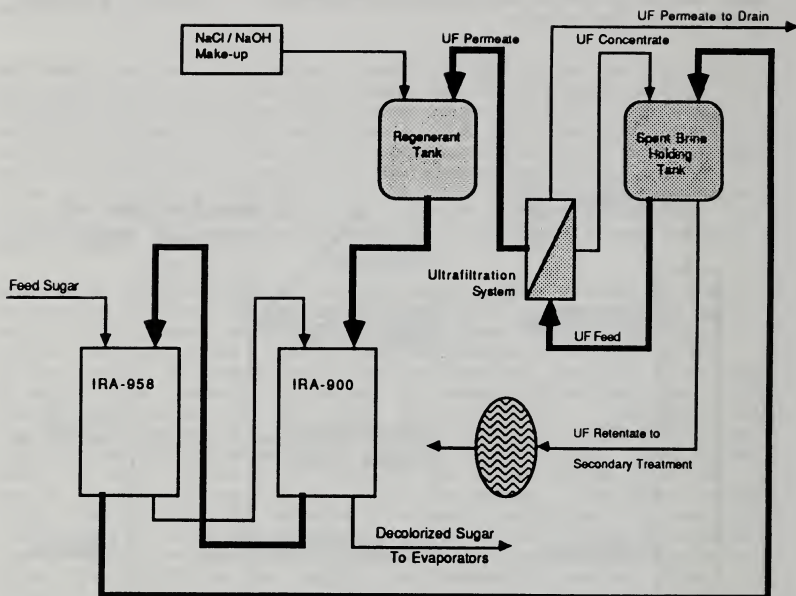
solution circulating throughout the system. Two schematic diagrams showing how this UF system operates are shown below in Figures 1 and 2. Other methods of filtration, such as reverse osmosis, fixed membrane and fixed media, tend to foul or exhaust very quickly due to the high concentration of organic material present in the brine.



Single Hollow Fiber Membrane
FIGURE 1.



Exploded View of Hollow Fiber Membrane Wall
FIGURE 2.



**Schematic Diagram of Ultrafiltration System
for Processing Spent Brine Regenerant**

Figure 3.

Shown above in Figure 3 is a schematic diagram which depicts how an ultrafiltration system would fit in with the overall sugar decolorizing operation. In this example, a chlorine dioxide system is shown as the secondary processing system.

There are several ways to operate an ultrafiltration system employed for this purpose. First, by way of example, let's assume we have one liter of spent brine at a 1,000 ppm concentration of carbon as carbon. The UF system can be permitted to run until 95% of the original volume is permeate and only 5% is concentrated retentate. This method of operation is the most simple and straight forward. The volume of discharge, as compared to no treatment, is virtually the same while the concentration of organic matter discharged is approximately one-half.

PERCENT COLOR REDUCTION IN PERMEATE AS A FUNCTION OF PERMEATE VOLUME

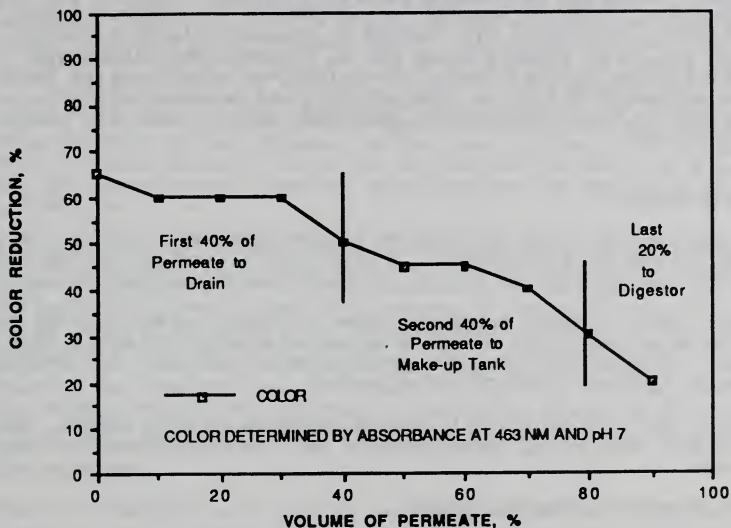


Figure 4.

A second mode of operation, as illustrated in Figure 4, is to segment the permeate during the run into three portions: one of the drain, one recycled as regenerant and one for secondary treatment. Again, using the same one liter of 1,000 ppm carbon solution as our example, the first 400 mL of permeate volume will go to the drain. This portion of ultrafiltration run represents the time in which the relative concentration of water in the permeate is highest and the concentration of organic matter is lowest. Generally, we observe an average reduction of 60% in organic carbon concentration during this portion of the run, as compared to the initial concentration of carbon in the UF feed, which is constantly increasing in concentration. (All of the values reported herein are based on the initial concentration of carbon in the UF feed solution.) This translates into 160 mg of carbon going to the drain. ($400 \text{ mL} \times 400 \text{ mg C}/1000 \text{ mL} = 160 \text{ mg C}$.) This represents an 84% reduction in organic matter discharged and a 60% reduction in the volume discharged, as compared to no treatment. The second 400 mL of permeate is sent to the regenerant make-up tank. In our example, this portion of the UF permeate contains approximately 220 mg of carbon, as the average reduction in carbon during this time is 45%. The volume will be returned to one liter, resulting in less than 90 ppm of carbon in the regenerant. Sodium chloride and sodium hydroxide will be added as necessary to restore the regenerant components to their original concentrations, 10% and 0.2%, respectively. The remaining 200 mL of solution, the retentate, which contains the

largest portion of carbon, 620 mg, is sent for secondary treatment. These systems typically oxidize or reduce the organic matter. Examples of these include, but are not limited to, ozonation, chlorine dioxide, and digestion by microbiological activity. Obviously, this method of operation is more complex than the first example where 95% of permeate was discharged; however, the system clearly shows distinct advantages in terms of the volume of discharge, concentration of organic material present in the discharge and the opportunity to recycle some of the brine.

EXPERIMENTAL

A Romicon Lab 1 Ultrafiltration Single Cartridge Unit was used in the laboratory experiments. A PM-5 ultrafiltration hollow fiber cartridge was used in all of the experiments reported herein.

Ten liters of spent brine was added to the UF reservoir for the purpose of providing enough solution to conduct an eight hour run. Permeate and retentate samples were collected for analysis during the operation of the UF system.

There were two analytical methods employed in this study. They were Gel Permeation Chromatography and Spectrophotometric Absorbance.

Gel Permeation Chromatography

The Gel Permeation Chromatography (GPC) analyses were performed to establish the molecular size of the color bodies present in the spent brine from columns of Amberlite IRA-958 and Amberlite IRA-900. This method was also used to analyze the ultrafiltration feed and permeate solutions. The GPC analyses were conducted under the following conditions:

Column:	ToyoSoda TSKgel G2500PWx1
Mobile Phase:	0.05M NaAc, pH 6.8
Flow Rate:	1.0 mL/min
Detector:	Refractive Index

The chromatograms of the spent brine from the columns of IRA-958 and IRA-900 are displayed in Figures 5 and 6, respectively. Figure 5, the brine from the acrylic based IRA-958, contains a high molecular weight component as seen by the elution peak at 56 counts. This high molecular weight peak is not present in the chromatogram for the IRA-900 brine regenerant. This two bed

* Amberlite is a registered trademark of Rohm and Haas Company.

system for sugar decolorization, Amberlite IRA-958 followed by Amberlite IRA-900, was developed for just this reason. The IRA-958 has a higher regeneration efficiency with respect to organic materials; therefore, it is placed in the primary position to remove the bulk of the sugar color. The styrenic based IRA-900, in the secondary position, serves as a polisher to remove the lower molecular weight species. Figure 7 is a chromatogram of a spent brine sample which passed downflow through the IRA-900 column and then through the IRA-958 column in the same manner. This solution is very typical of the spent brine which is the starting feed solution to an ultrafiltration system. As expected, the chromatogram contains both high and low molecular weight components.

Figure 8 is a GPC chromatogram of permeate solution which has passed through a PM-5 cartridge. A PM-5 cartridge has a nominal molecular weight cut-off of approximately 5,000 daltons. The chromatogram clearly shows the absence of the high molecular weight peak at 56 counts and a significant portion of the low molecular weight profile between 60 and 80 counts. This is significant from the standpoint that not only has the UF process reduced the concentration of organic matter for disposal, but it has selectively discharged the low molecular weight population. The general appearance and color of these samples to the naked eye are coffee black for the feed solution and medium yellow for the permeate.

Dextran standards were used to standardize the GPC instrument.

Spectrophotometric Absorbance

The spectrophotometric analyses were performed with a Hach DR/3 unit using a flow-through one centimeter cell. Deionized water was used to zero the instrument and all necessary dilutions were made with the same. All samples were adjusted to pH 7 prior to analysis.

CONCLUSIONS

Laboratory results indicate that the Romicon Ultrafiltration process for the treatment of brine regenerant from ion exchange materials employed to decolorize cane sugar can be used to assist in the management of these waste streams. This is accomplished by reducing both the volume and concentration of material for disposal.

RECOMMENDATIONS

Significant time and energy have been spent in the laboratory developing the application of this separation technology, Romicon Ultrafiltration, for use in the sugar industry. It is recommended that the ultrafiltration waste management system be evaluated through one-site pilot plant studies.

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Figure 5.
GPC Chromatogram of Spent Brine from IRA-958

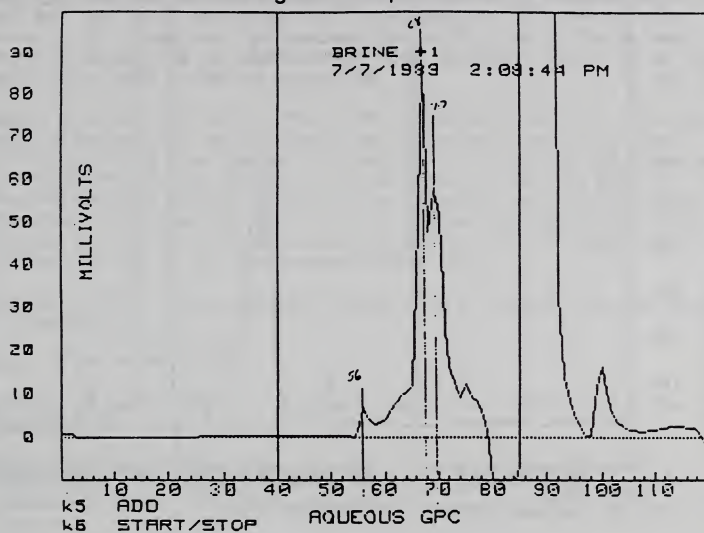


Figure 6.
GPC Chromatogram of Spent Brine from IRA-900

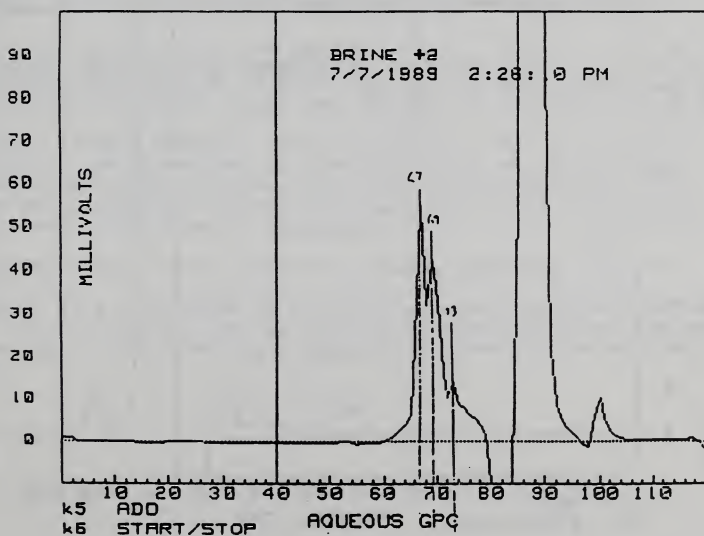


Figure 7.
GPC Chromatogram of Spent Brine from IRA-958 and IRA-900

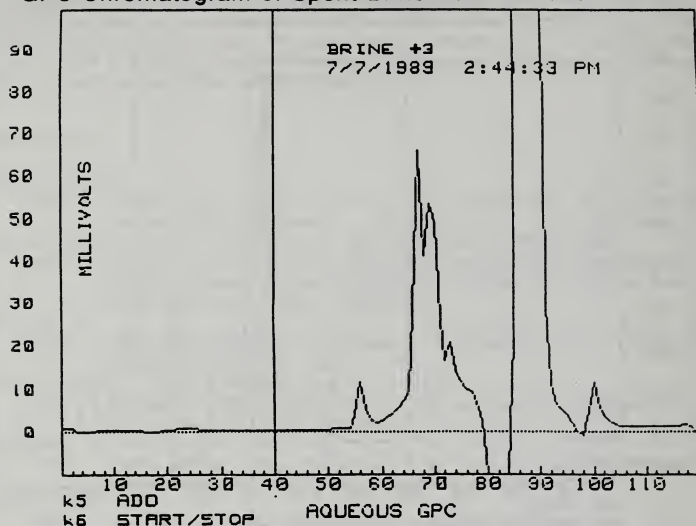
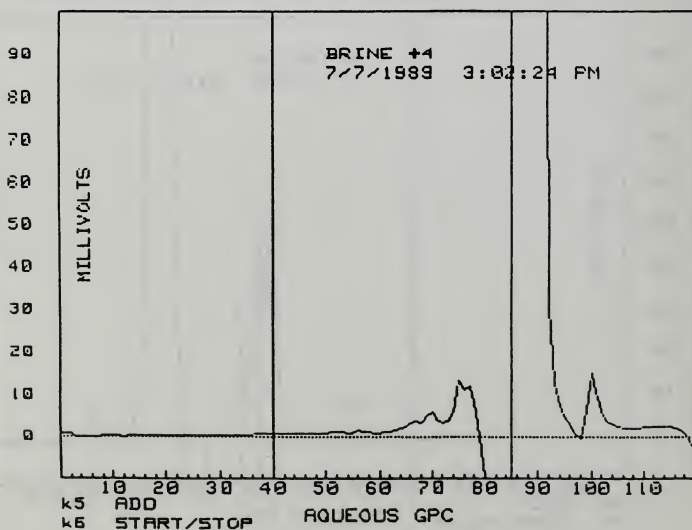


Figure 8.
GPC Chromatogram of UF Permeate Through a PM-5 Cartridge



DISCUSSION

Question: One of the historic problems with any membrane process (UF or RO) has been progressive, irreversible fouling of the membrane, and subsequent flux decrease with time. Would you comment on that - if you've solved this problem, how you've done it, and with what cleaning schedule.

Wilson: A good question. The great advantage of this UF system is that cleaning can be achieved in two ways: one is by back flushing, running the system in reverse to remove materials that have collected on the membrane. The other is to run sodium hydroxide through the UF system as a cleaning and sterilizing procedure. The chemical cost of the sodium hydroxide is essentially zero, because you can run the sodium hydroxide through the UF system and on in to the regeneration tank.

Question: Today then, you have been able to stabilize the flux with cleaning.

Wilson: That's correct.

Question: I don't believe you mentioned the relationship between temperature and flux rate.

Wilson: As the temperature increases, your flux rate will increase too. It's advisable to run the spent brine through the UF system in short order, to get optimum energy utilisation from the system.

Question: Any effect with the higher temperatures on chemical degradation of the membrane?

Wilson: No, the polysulfone membrane is thermally stable to 75°C.

Question: This is a nice approach to reduce waste. If you were to combine this with Mr. Bento's approach to regenerant recovery, in the previous paper, would that produce an even better system?

Wilson: I think that's a logical approach. By incorporating as many ideas and approaches as possible, we'll be able to meet increasingly tight government and regulatory sanctions. This could be a secondary treatment to turn color into a solid waste, and therefore more easily disposable.

Question: What is the lifetime of these membranes?

Wilson: It's a function of use, but we expect them to run from 3 to 5 years at least. We use them in other applications and have found that 3 to 5 years is a typical lifetime in some applications.

REMOVAL OF AMMONIA FROM CONDENSATES AND SURPLUS CONDENSER WATER BY STRIPPING WITH AIR

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Südzucker AG Mannheim/Ochsenfurt

The wish to recover the free ammonia appearing in the beet processing is an old one in the sugar industry. So reports Wohryzek in 1928 in his Handbook "Chemie der Zuckerindustrie": "The endeavour to recover at auxiliary plants the ammonia which is set free in the factory at different stages, exists since the year 1868" (Wohryzek, 1928). The amount of ammonia which could be recovered from the condensate of the evaporator station but also from the vapours of the carbonation vessels was found to be about 150 g per t of beet. Wohryzek had already realized that the ammonia stems from the saponification of glutamine and asparagine. Wohryzek closes his report with a note of Procházka, "that today there is not much hope that the ammonia in the sugar factory could be recovered successfully and profitably".

Topics like environment, pollution or waste water treatment for ammonium components were not that important at that time.

Up to some years ago one was interested in α -amino nitrogen compounds, i.e., free amino acids and the amides glutamine and asparagine, mainly because of technological considerations.

Some investigations had shown (Schneider et al., 1959; Schiweck, 1967; Kubadinow et al., 1975 and Reinefeld et al., 1982), that about 95% of glutamine and about one third of asparagine are saponified during juice purification and in the initial stages of the evaporator. The saponification of glutamine results in the generation of ammonia and pyrrolidone carboxylic acid whereas asparagine leads to ammonia and aspartic acid. The extent of amide saponification in the initial evaporator stages is determined by physical parameters, e.g. temperature, residence time and pH value in the juice purification. As shown in figure 1, only a partial saponification to the extent of 60 - 80% can be achieved under the conditions of currently employed juice purification processes (Schiweck, 1967; Bohn et al., 1985). Therefore we had proposed some years ago (Schiweck, 1976) to bring the saponification to completion in thin juice 1 by keeping it at 120°C for about 10 - 20 minutes and to distill off the ammonia from the juice prior to evaporation.

If it is in the evaporator that the ammonia is liberated, then the pH value of the juice can fall to an extent to cause the generation of invert sugar. The invert sugar itself as well as its degradation products react with amino acids present in the juice to give rise to Maillard products and - corresponding to

Strecker degradation - to aldehydes and pyrazines (Eichner, 1982). This is accompanied by an increase in the colour of the juices. Juices exhibiting a pronounced decrease in pH and increase in colour are characterized as thermo-unstable.

How far the sucrose recovery is influenced by the amino acid content of beet (α -amino-N content) hasn't found a universally accepted answer yet. The various recovery formulae differ in their valuation of the molasses forming properties of amino acids (Reinefeld et al., 1986).

Nevertheless it is being tried to reduce the α -amino-N content of beet through breeding as well as fertilizer application based on soil analyses - this later measure having the additional bonus of alleviating the nitrate load on soil and ground water (Ziegler, 1989).

Figure 2 showing the average α -amino-N content of beet grown in Southern Germany doesn't conclusively show whether these measures have already been effectual. It is to be hoped that these measures may stabilize the α -amino-N content of beet at a low level or may even lead to a further decrease during the coming years.

That the α -amino-N content of sugar beet is determined by the growing area and the weather conditions prevailing during the individual growing periods, can be seen from figures 3 and 4. In figure 4 it can also be seen that regions with lower α -amino-N content of beet don't show such high variation from year to year as those with relatively high α -amino-N content. The frequency distribution of α -amino-N content in beet from individual deliveries is also different from the two regions (figure 5): it is broader in the case of the regions with high α -amino-N content whereas it resembles a Gauss-distribution for the region with low α -amino-N content.

Now, as shown in figure 6, there is a high correlation between α -amino-N content of beet and the ammonia content of condensates. Therefore in a sugar factory one has to reckon with varying ammonium contents in condensates and waste water too as the ammonium content of waste water of a sugar factory stems mainly from the condensates.

In West Germany recent waste water regulations restrict the amount of ammonia-N to a maximum of 10 mg/l in the effluent. In the sugar industry a limit on all the nitrogen compounds of the waste water is expected; the reason being the high nutrient content in the waste waters, which may in turn contribute to the pollution of the North and Baltic Sea.

Today the common technique for removal of ammonia in the waste water is the biological nitrification (figure 7). Biological nitrification is the term used for the bacterial process in which ammoniacal nitrogen is oxidized to nitrite (reaction 1), and then to nitrate (reaction 2). This process is carried out in an activated sludge plant in which the bacterial culture in the form of a floc is fed with the waste water, into an agitated, aerated tank. The biological process is however very sensitive to variations of temperature or loading in the plant. Furthermore the plant produces excess-sludge which must be disposed of.

In view of the taxes to be paid for the inorganic nitrogen in the waste water, as to be expected in West Germany after 1991, the transformation of ammonia to nitrate alone is not reasonable. That means, the biological process must contain a denitrification step too, so that the cost of the waste water plant would be higher (figure 8).

In the biological denitrification step the nitrate is reduced in the presence of organic matter to molecular nitrogen. Therefore in a sugar factory, ammonia should be removed at the source before it appears in the waste water. This can be done as mentioned earlier or by stripping of the dissolved ammonia from the condensate or surplus condenser water. First we carried out trials in the 1985 campaign with a pilot stripping plant designed for a low rate of about 1 m³/h. Air was used as the entrainment gas. The stripping efficiency of the ammonia depends upon the pH, the temperature of the treated water and the air: water ratio (m³/m³).

The trials were performed at various pH-values from 9 - 11 and temperatures ranging from 40 to 70°C. The target ammoniacal nitrogen content of the effluent was a maximum of 2 mg/l corresponding to a removal of about 99%. Figure 9 shows ammoniacal nitrogen content of treated condensate with a pH ~ 10, measured at 20°C, as function of stripping temperature and the air: water ratios. The results of the trials demonstrated many advantages of the stripping process: it is easy to handle; it doesn't need an adaption time; it produces a utilizable product namely ammonium sulfate or ammonium phosphate and there are no irreversible or long-lived troubles of the process.

As a result of these trials Südzucker decided to install such stripping equipment in the Offstein factory for the 1989 campaign.

The stripping plant is designed for a flow rate of 400 m³/h, an ammonia content in the influent of 150 mg N/l and 1.7 mg N/l in the treated water at a process temperature of 55°C. The air requirement under these conditions is 320,000 m³/h. (ratio water : air as 1 : 800). The total energy requirement is 300 kW.

Figure 10 shows the schematic diagram of the ammonia stripping plant at the Offstein factory.

The plant consists of a stripping column, an absorption column, a closed circuit for the air and a circuit for the ammonium salt. Each column contains a packing material having a large contact area between liquid and gas phases.

After alkalization with NaOH-solution, condensate flows into the stripping column, passing downwards through the packing material. Air is passed countercurrent through the liquid phase. The air, enriched with ammonia, is blown upwards into the absorption column, against a countercurrent flow of acidified $(\text{NH}_4)_2\text{SO}_4$, so that the ammonia is eliminated from the gas phase. The exhaust air is re-used for stripping. The operation of the process is isothermic; actually there is a slight temperature increase due to the heat of neutralization of the ammonia. The concentration of ammonium sulfate in the obtained solution ranged between 29,3 and 47,9% (average 38.3%).

The plant was put on stream on 20th October 1989. In this first campaign we tested two different operating conditions (figure 11):

- From 20th October to 31st October and from 18 November to the 19th December the plant was fed with surplus condenser water.
- From 1st November to 17th November we treated condensate in the plant, before introduction into the condenser water circuit.

Figure 12 shows the variation of flow rates through the plant and process temperatures tested.

Treating condensate for the 6th to the 8th week the average flow rate was 433 m³/h for total-condensate and 304 m³/h for surplus condensate. In case of surplus condenser water treatment the average flow rate was 262 m³/h. This figure shows also the temperature of the process. It ranges from 61 to 69°C (average 62°C) treating condensate, and from 42 to 46°C (average 44°C) in case of surplus condenser water.

The pH in the influent and effluent of the plant is shown in figure 13. In the most cases the pH of the treated water was 10.1 (measured at 20°C).

The ammonia contents in the influent and effluent of the plant are shown in figure 14. In the feed the ammonia content ranges from 99 to 153 mg N/l (average 131 mg/l), in the stripped water from 0.9 to 3 mg N/l (on average 2.0 mg N/l). For the treatment of 1 m³ condensate 240 g NaOH and 430 g H₂SO₄ was consumed.

The plant shows considerable process stability.

Now it would be interesting to know the ammonia content achieved in the treated waste water after stripping of surplus condenser water.

At the Offstein sugar factory the anaerobically digested water (Schiweck et al., 1985; Nöhle, 1990) and the surplus condenser water are treated by lagooning in three aerated ponds (figure 15). After treatment the cleaned water flows together with cooling and sealing water into the river. The flow rate through the anaerobic plant ranges between 49 and 92 m³/h (average 76 m³/h). The flow rate on surplus condenser water is about 253 m³/h (range: 227-276 m³/h). In the anaerobic plant some ammoniacal nitrogen is generated through the degradation of the organic nitrogen compounds.

Figure 16 shows the ammonia content in the influent and effluent of the anaerobic plant. In the waste water to be treated anaerobically the ammonia content is up to 77 mg/l. At the Offstein factory excess condenser water with an ammonia content of about 130 mg/l was used to fill up the beet wash water circuit. The ammonia content in the inlet depends on the ammonia content of the flume and beet wash water so that the ammoniacal nitrogen in the effluent is up to 94 mg/l, showing that the average anaerobic production of ammoniacal nitrogen ranges from 15 to 20 mg N/l (difference between influent and effluent of the plant). This amount of generated ammonium must be taken into consideration when the ammonium concentration in the discharged water from a factory is calculated.

The stripping plant was put on stream in the 5th week of the campaign and so there was a preliminary ammonia load in the water circuits. In the inlet of the first lagoon the ammonia content was lower than 25 mg N/l already in the 10th week (figure 17). The ammonia content in the discharged effluent was lower than 10 mg N/l from the 12th week on, because of the long retention time in the lagoons of 12 to 14 days.

Now what is planned for the next campaign to ensure an ammonia content of less than 10 mg/l in the discharged water?

1. The stripping plant shall be put on stream right at the beginning of the campaign, so that there is no build-up of the ammonia contents in the water circuits.
2. Condensate from the carbonation vapours shall also be treated in the stripping plant.
3. Only pre-treated condensate will be used in the production process in the sugar house as well as the extraction system.

4. Stripped condensate shall be used to make up the flume and wash water circuit.

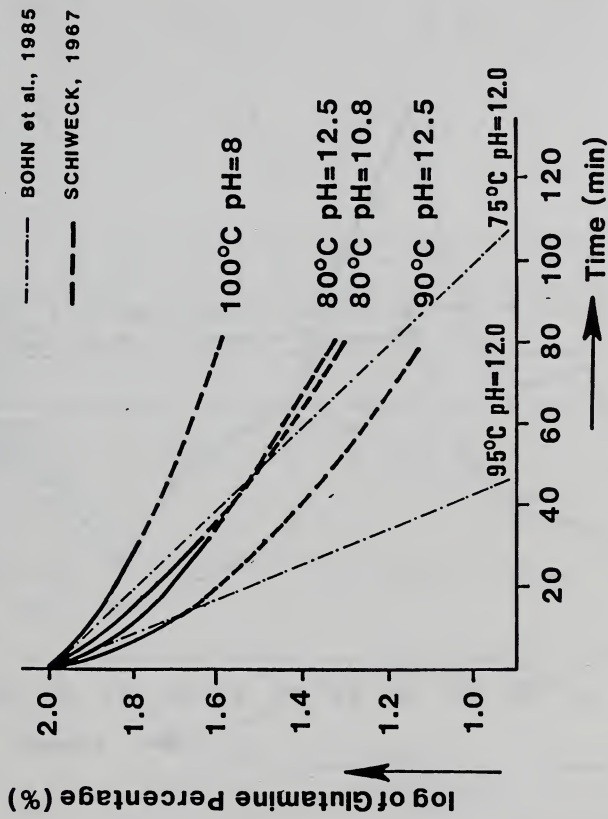
So we expect also low ammonia contents from the anaerobic plant. Only when the stripping plant has been in operation for at least one full campaign and when some additional aspects like ammonia generation in the anaerobic treatment plant as well as the utilization of the ammonium salt produced have been fully clarified, can we be in a position to say whether for the sugar industry this technology is preferable to standard processes or not.

Moreover the special features of the Offstein factory must be stressed, since as shown above, they are not typical for a sugar factory.

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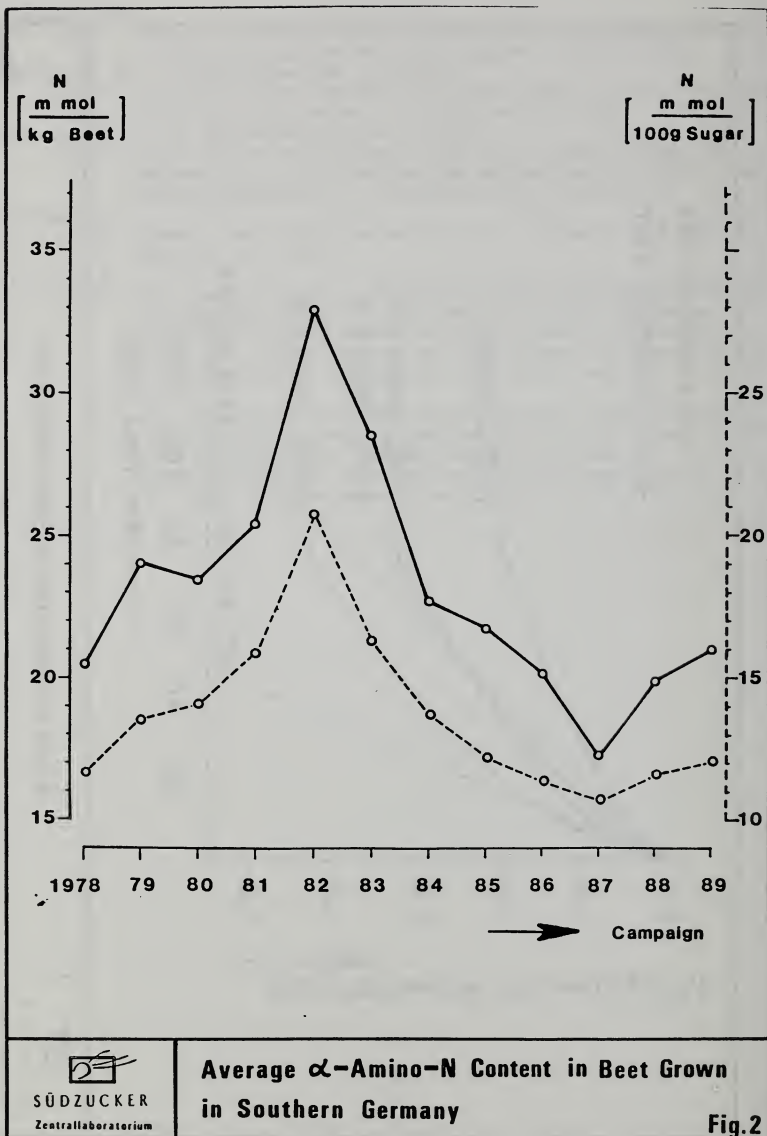


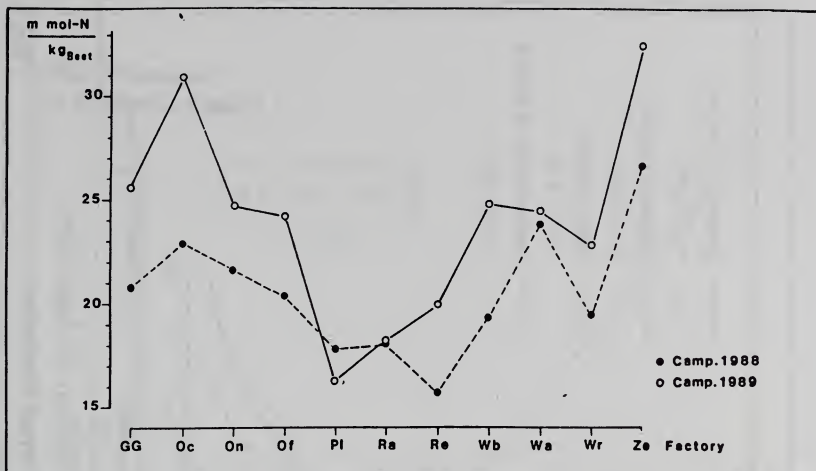
Rate of Glutamine Saponification

Fig. 1



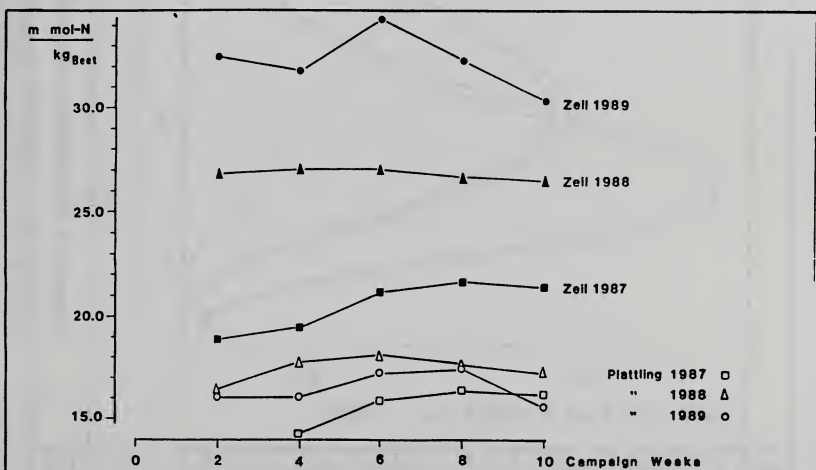
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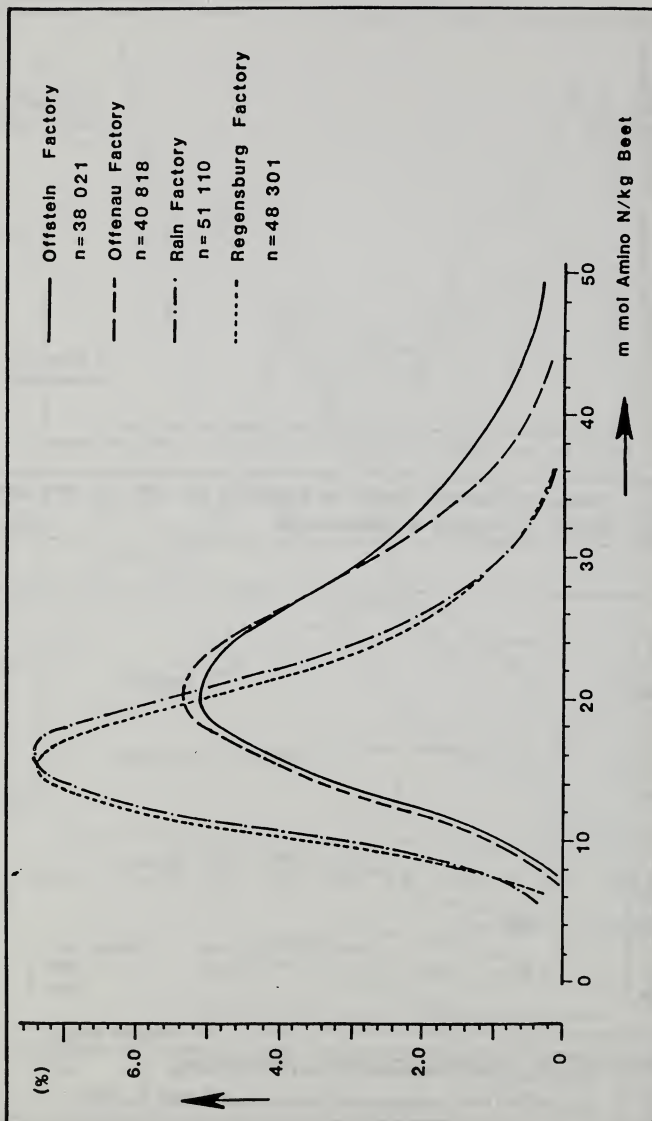
Average α -Amino-N Content in Beet During the 1988 and 1989 Campaigns at the 11 Factories of Südzucker-AG

Fig.3



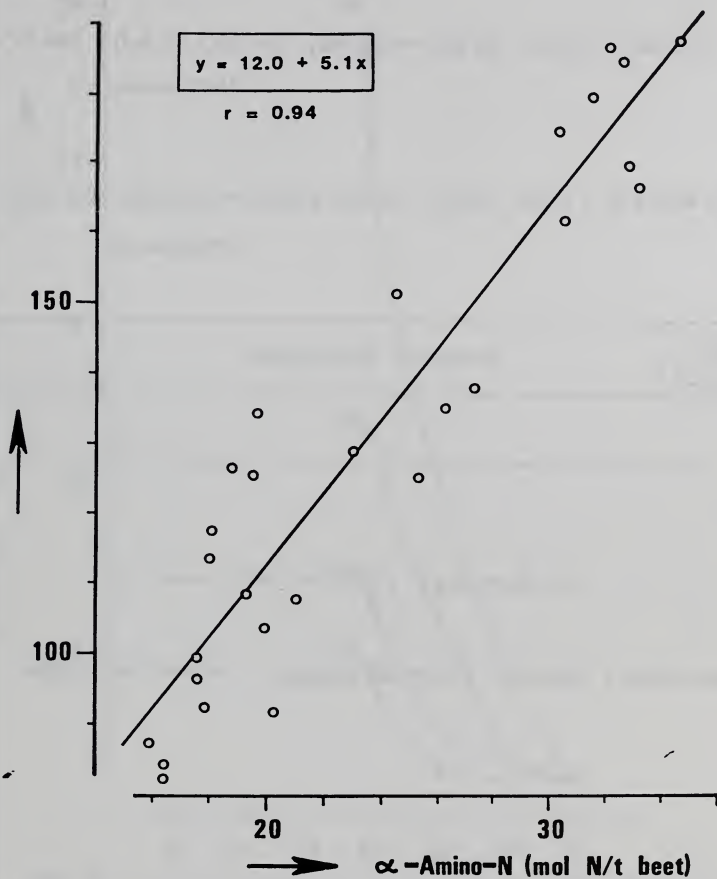
Time Course of α -Amino-N Content of Beet During the 1987-1989 Campaigns at the Plattling and Zeil Factories

Fig.4



Frequency Distribution of α -Amino-N Content in Sugar Beet
During the 1989/90 Campaign in Different Factories

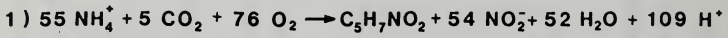
**NH₄-N-Content
in Condensate (mg/l)**



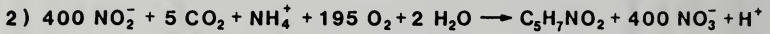
SÜDZUCKER
Zentrallaboratorium

**Relationship between α -Amino-N Content in Beet
and NH₄-N-Content in Condensate
(1989/90 Campaign)**

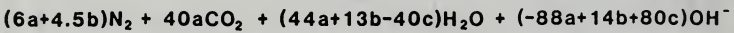
Fig.6



(Nitrosomonas)



(Nitrobacter)



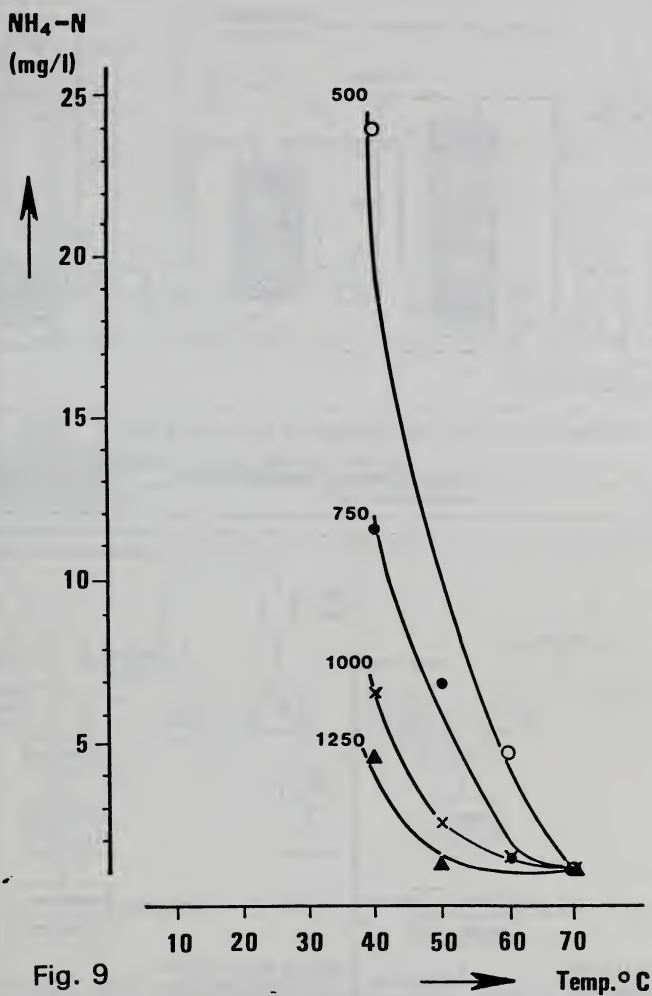
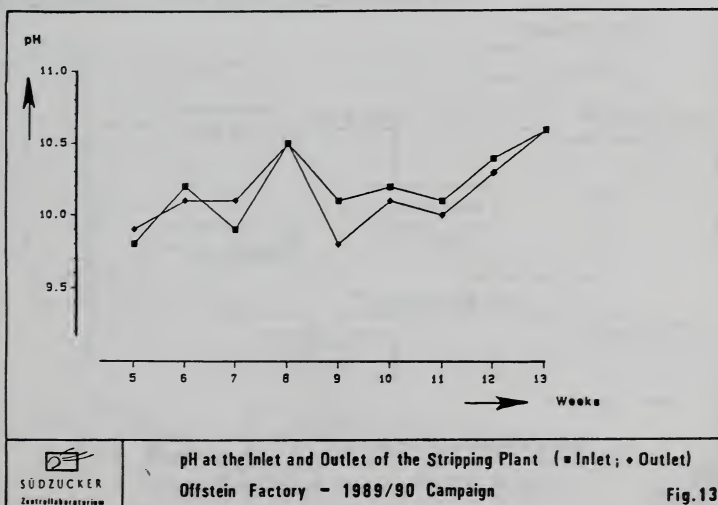
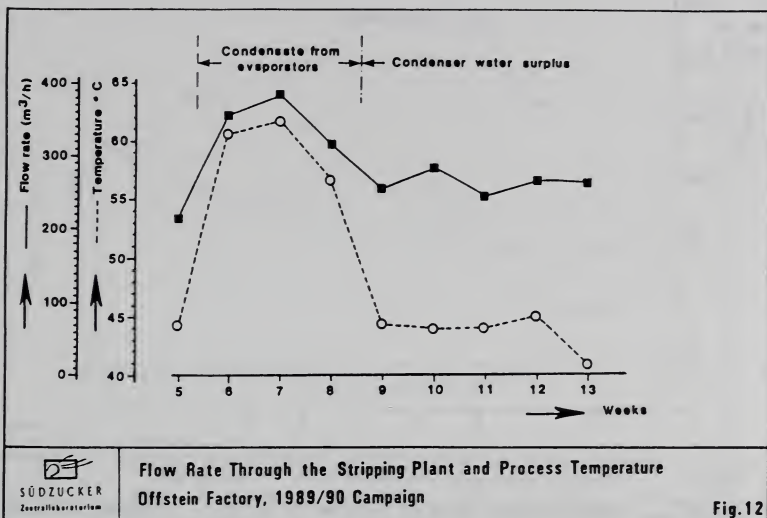


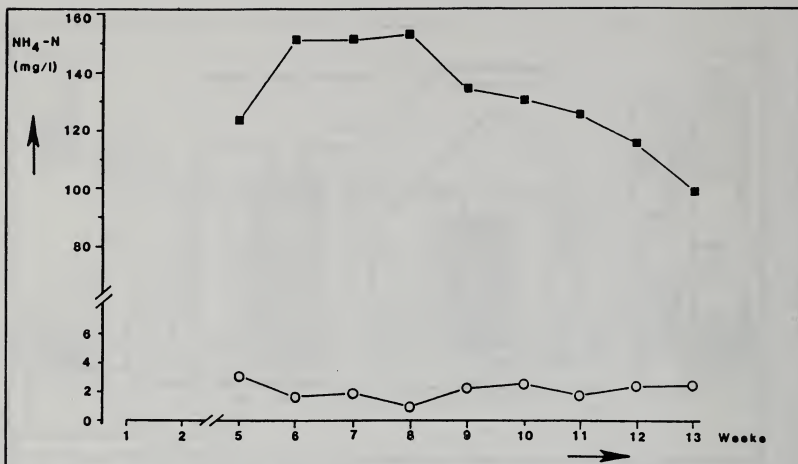
Fig. 9



SÜDZUCKER
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Ammonia Contents in the Effluent at Different Temperatures and Air: Water Ratios (m³/m³) at pH 10.0





Efficiency of Ammonia Elimination in the Stripping Plant

Offstein Factory, 1989/90 Campaign — ■ Inlet; ○ Outlet

Fig.14

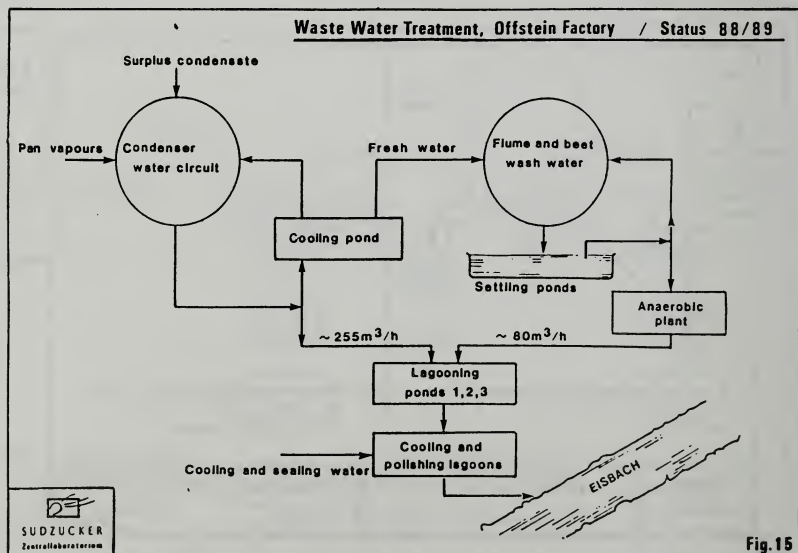
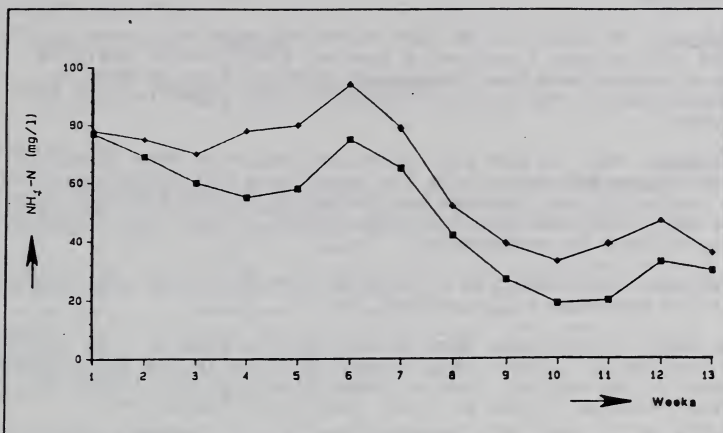
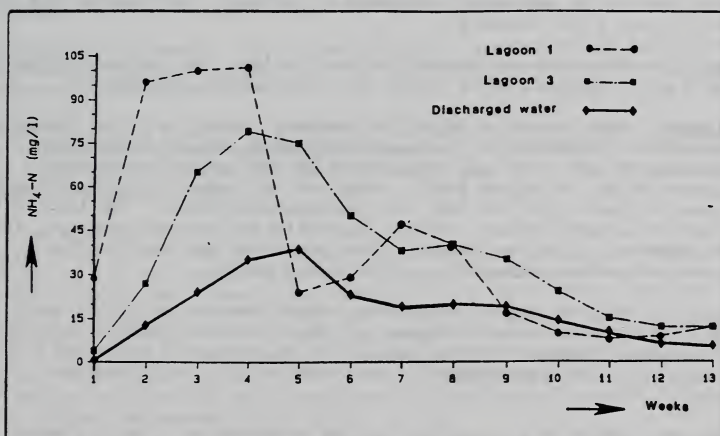


Fig.15



Ammonia Content at the Inlet and Outlet of the Anaerobic Treatment Plant, Offstein Factory, 1989/90 — Inlet ■, Outlet ♦ **Fig.16**



Ammonia Contents in the Lagoons and in the Discharged Water into the River: Offstein Factory - 1989/90 Campaign **Fig.17**

DISCUSSION

Question: It seems to be that there are some very good conditions here to use a weak-acid type of ion-exchange resin to remove ammonia and then regenerate with acid to produce an ammonium salt. Has any consideration been given to this approach?

Schiweck: Yes, we had full installations with this system at three of our factories. In our experience, this did not provide the best solution, because the formation of the ammonium salt, from materials absorbed from condensate, was not satisfactory - it's too much of a mixture.

Question: I believe you can add acid to the spent regenerant and build up ammonium salts in that way.

Question: I know your company has been working on this problem for many years - it's a very topical problem in the EC. Having regard to the capital cost of their plant, do you intend to install similar plants at other Suedzucker factories?

Schiweck: Yes - the capital cost is about 6 million Deutsche-marks. You recover a lot of energy cost. Further installation depend on the rulings of authorities for the content of ammonium in the discharged water. We can remove, when we treat all our condensate, 99% of the ammonium from the factory, but you have ammonium also in the sugar house, in vapors and condenser water. Therefore, it is necessary to make a balance, and choose the best solution for your factory.

Question: You spoke of water to air ratio. Do you also have a water to air surface area ratio for the actual strippers?

Schiweck: Yes. In the Offstein factory there is a low temperature drier. Heated air is needed for this drying equipment; therefore, there is a pre-condensor in the sugar house that produces water at about 60°C. This water, mixed with condensate, is used to heat air for the low temperature drier. We tested the equipment in two places; first, directly in the condensate, after heat exchange with raw juice. The second place was in the circuit of condensate and precondensate water.

Question: Concerning organic compounds present with the ammonia in the condensate: what happens to the C.O.D.? Does it remain in the condensate after stripping, or is it stripped together with the ammonia, and subsequently adsorbed in the acid washing column?

Schiweck: The C.O.D. remains in the condensate. Only a small amount of aldehydes go into the ammonia solution. The C.O.D. content of the ammonia solution is about 100 mg/l.

ALKALINE DIFFUSION

N.F. Okojie and D. Sargent

British Sugar Research and Development

INTRODUCTION

For at least eighty years, numerous workers have considered the possibility of pretreating beet with lime before extraction (Weinrich, 1905, 1908 and 1910; Borghi, 1946; Degtyar, 1948; Bonelli, 1959; Loof et al.; Susic, 1959, Bobrovnik et al., 1977 and Vukov et al., 1973). In many cases the objective of such work has been to improve the separation of water from the exhausted pulp. A consequence of such treatment would be the possibility of raising the pH of diffusion into the alkaline region. This concept has become known as alkaline diffusion and its implementation would represent a major change in processing methodology for the beet industry.

The standard method for extracting sugar from beet is by counter-current diffusion with hot water, carried out under slightly acidic conditions caused either by natural thermophilic bacterial activity or, preferably, by the addition of acid to the diffusion water and into the diffuser body (Oldfield et al., 1977). Such conditions prevent alkaline degradation of the beet pectin, due to the presence of ammonia in diffusion supply water, and the consequent softening of the pulp (Carruthers et al., 1956).

Following diffusion the extracted pulp is pressed in twin screw continuous presses before drying and sale as animal feed with or without molasses addition.

Because of the high fuel costs of drying pulp and the increasing environmental pressures surrounding pulp drier odour emissions it is imperative to achieve as high a pressed pulp dry substance as is economically possible.

Since the mid-1950s salts of polyvalent cations, particularly calcium or aluminum, have been added to the diffusion supply water to act as pressing aids. They are believed to increase pulp rigidity by cross-linking carboxylic acid sites on the beet pectin (Shore et al., 1983). Calcium chloride was used by British Sugar since the 1956/7 campaign (Carruthers et al., 1957), and following factory trials in 1981/2 (Shore et al., 1983) we have changed to the use of gypsum (mineral calcium sulphate dihydrate) as this gives a significant benefit in terms of reduced molasses formation.

Current technology, including the use of pressing aids with hollow, perforated, press spindles allows a modern factory to achieve about 32% pulp dry substance for much of the campaign.

Although there has been research into improved press design such as the hyperpress of Generale Sucriere, ENSAM-ESERAM and ENSIA (Pouillade et al., 1987) and into novel technology such as the application of electroacoustic dewatering from Battelle (Muralidhara et al., 1986; Muralidhara et al., 1987) there is a yet no cost effective, developed, method of routinely achieving higher pulp dry substance.

Research into the application of alkaline diffusion following a pretreatment of cossettes with lime has been seen by many workers as a route towards higher pulp dry substance.

The reaction of polyvalent cations with beet pectin is normally regarded as an ion exchange reaction (Shore et al., 1983). This approach suggests that increased benefits might be obtained if the beet pulp is treated with the calcium ions as early as possible in the diffusion process, preferably under alkaline conditions to increase the uptake of calcium ions relative to hydrogen ions on the carboxylic acid groups.

But can an alkaline treatment of beet material ever be successful when the concept of an alkaline diffusion appears to be in direct conflict with best modern practice in the beet industry? In this paper we shall try to show that there are conditions under which such a treatment is possible, what benefits might be achievable and where the state of the art lies following the 1989/90 campaign.

THEORETICAL CONSIDERATIONS

It is relevant to consider the overall composition of the organic structural material of beet tissue in order to understand the role played by each of the components.

Composition of beet marc

The three major constituents of beet marc are cellulose, hemicellulose and pectin whilst lignin, protein and ash are also present in small amounts. Buchholz in his introduction to a paper on the chemical aspects of the mechanical dewatering of pulp quotes the data in Table 1 (Buchholz et al., 1986), which is closely similar to that of Michel and Thibault (1988) except for the lignin content (2 to 7% versus 1 to 2%) which may turn out to be more dependent on the maturity of the samples analysed than is the case for the other components.

Table 1: Composition of beet marc, percent on total solids

Cellulose	21 - 27	Pectin	17 - 25
Lignin	2 - 7	Protein	5 - 9
Hemicellulose		Sugar	1 - 2
- Araban	20 - 22	Ash	3 - 7
- Galactan	6 - 7		

Cellulose, a main structural component of plant tissue, is inert under the normal processing conditions and also those of alkaline diffusion.

Lignin, which may have an important role in the mechanical properties of fresh beet, is at a low level in beet when compared to many plant materials and is considered to be unreactive except under very aggressive conditions.

Hemicelluloses, by original definition those materials which could be extracted from plant tissues by aqueous alkaline treatment, are not chemically related to cellulose but are found in close physical association with it in both primary and secondary cell plant walls (Whistler et al., 1970).

In the secondary cell walls the hemicellulose is found bonded to the pectin backbone. However when estimates of hemicellulose content are made by conventional methods there is usually no differentiation between source location and the value quoted will include the material which was originally part of the pectin structure.

Two types of hemicellulose can be extracted from sugar beet tissue: one is a polymeric material, known as arabinan or araban, where the major monomer is L-arabinose whilst the other, known as galactan, is a polymer which is largely D-galactose.

Although these materials are easily extracted under very alkaline conditions we have no evidence that they play an important role under the conditions proposed for alkaline diffusion unlike the remaining materials, pectin and protein.

Structure of beet pectin

Beet pectin consists of a linear polymer back-bone of alpha-(1-4)-linked D-galacturonic acid units, interrupted at intervals of approximately 25 monomers by rhamnose molecules.

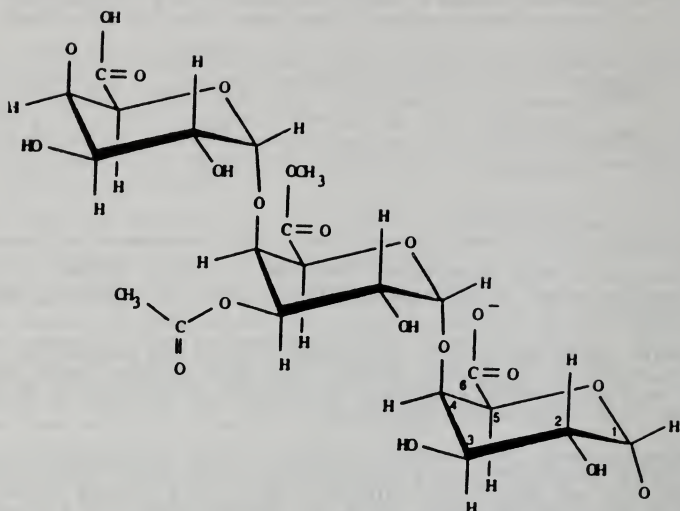


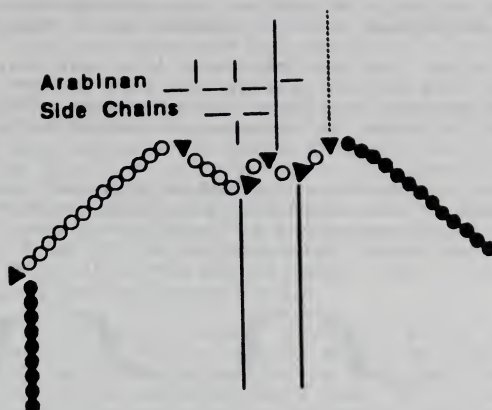
Diagram 1. The fundamental polygalacturonate chain structure.

In beet pectin the galacturonic acid is methylated at more than 50% of the carboxyl sites and also carried acetyl groups at many of the C2 or C3 sites (Diagram 1) and is in this latter respect different from most other pectins which have few or no acetyl groups present.

Determination of the O-acetyl content of beet pulp gives a value of about 4% so that if beet pulp is about 25% pectin and all the O-acetyl is in the pectin, then O-acetyl must be about 16% on pectin. This suggests one acetyl group for every monomer on average (McCreedy et al., 1966; Dea et al., 1986).

The L-rhamnose molecules which interrupt the polygalacturonate chain form the branch points at which side chains of arabinose and galactose polymers are attached. These side chain are themselves highly branched and have been described as the "hairy" regions of the pectin polymer.

Diagram 2, based on that in a comprehensive review of pectin structure (Jarvis, 1984), attempts to show how the branched and linear regions relate and how the methylated and unmethylated linear regions may be separated by rhamnose units. In pectins with a very high degree of methylation such separations may not be so precise.



- Non - esterified galacturonan block
- Methyl - esterified galacturonan block
- ▶ L - rhamnose

Diagram 2. Methylated and unmethylated regions of beet pectin.

The degree to which the pectin part of the sugar beet reacts during either normal or alkaline diffusion can play a significant role in the success of the operation.

Reactivity of beet pectin

Even though beet pectin is insolubilised by its bonding to the other major cell wall components it has chemical reactivity in three different ways:

- i. by simple ionic exchange mechanisms
- ii. by de-esterification at C5 and de-acetylation at C2 or C3, releasing methanol or acetic acid respectively
- iii. by beta elimination resulting in cleavage of the 1-4 glycosidic linkages.

i. Ion exchange reactions

The non-esterified carboxyl groups on the pectin backbone behave in a very similar manner to conventional ion exchange resins with a total ion exchange capacity of at most 4.5 meq/100g beet (Buchholz et al., 1986).

In fresh beet material about half of this capacity is already occupied by calcium, potassium and sodium, the remainder being protonated. Only relatively weak solutions of the major cations of beet processing need to be used to saturate the ion exchange capacity of the carboxyl groups. Buchholz (1986) reports that a

0.01 normal solution of calcium will do this above pH 4 but that at lower pH, protons compete effectively for the active sites.

When polyvalent ions are introduced into beet pulp, replacing the monovalent ions already present, the pulp becomes structurally firmer and presses to a higher dry substance. This effect is considered to be due to increased cross-linking between pectin chains.

Morris et al., 1982 have proposed an "egg box" model for the conformation of the fundamental poly-galacturonate chains when they are closely associated as in gels or films. This model, based on experimental determination of the quantity of calcium which can be tightly bound to the pectin is shown in Diagram 3.

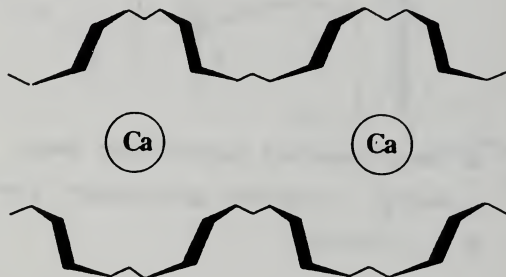


Diagram 3. Egg box model of aligned polygalacturonate chains held in close association by calcium ions.

In this situation two polymer chains are parallel aligned with calcium ionically bound between the free carboxyl sites. The conformation of the chains ensures that 50% of the carboxyl groups face outwards and are only loosely bound by either calcium or by monovalent ions. Thus when the degree of calcium binding is determined, by establishing how much calcium can be removed by an excess of a monovalent cation, 50% is easily removed and 50% remains tightly bound.

In transposing this model to the in-vivo situation with sugar beet pectin, the presence of methoxyl groups reduces the number of available sites whilst the presence of acetyl groups prevents access, by steric hindrance, of ionic species.

The "egg box" effect raises the possibility that the exhausted pulp could be made to have an even higher exchange capacity if the esterified carboxyl groups could be de-esterified and made

available. The consequence might be that the pulp could then accept more polyvalent ion, such as calcium, and become firmer, tending to the "egg box" structure and pressable to greater dry substance.

In terms of improved pulp pressing, the ionic reactivity of pectin is the major factor. Increasing the number of available carboxyl groups may enable us to modify this reactivity in a favorable way and this view is supported by our experimental work. However the other types of chemical reactivity possessed by pectin must be considered carefully in order to understand the constraints to chemical modification of the properties of beet pectin.

ii. De-esterification and de-acetylation

The methyl group at C5 and the acetyl groups at either C2 or C3 of the galacturonate unit can be removed easily by alkaline treatment. The de-methylation reaction is of primary importance and Randall (1981) showed experimentally that the time needed to reach a steady state for the reaction of pectin, in vivo, with for example dry calcium oxide powder, was about 20 minutes and the reaction was 50% completed after six minutes.

In contrast to slow reactions cossettes immediately become rigid and yellow/green in colour immediately when contacted by the combination of hydroxide and calcium ions. Whilst we consider the formation of the yellow/green colour to be of no significance to the chemistry of alkaline pre-treatment of beet, being just a response of a chromophore to the high pH, the hardening of the tissue is of great interest. We have shown by simple experiments with, for instance, calcium chloride solution and sodium hydroxide solution, that the presence of both alkali and calcium are necessary to cause this hardening but that it does not matter in which order the tissue is treated with these reagents. The probable explanation of this effect is that the hardening is due simply to bridging, by calcium, of existing un-esterified carboxyl sites may available due to deprotonation in the alkaline medium.

Therefore the immediate and readily observable behaviour of cossettes when treated with lime probably does not involve the de-methylation reaction but, instead, this reaction continues slowly, during the pre-treatment period, creating potentially important sites for further calcium binding, whilst protecting the pectin from further degradation in the manner to be described below under glycosidic cleavage.

Randall (1981) showed that the de-acetylation reaction proceeds at about one third the rate of de-methylation, releasing acetate into the juice. This effect has probably no value to the sugar

extraction process as acetate is not removed during juice purification and therefore contributes to increased molasses production whilst also representing a small loss of pulp material. In addition it captures a significant part of the calcium oxide used in the cossette treatment. Tiszavary and Javrinecz (1962), however, report an increase in the rate of low grade sugar boiling in the presence of acetate.

The to which these reactions are allowed to proceed could be of great importance in the development of this technology, although at present the real target parameters are matters of hypothesis rather than fact.

In summary de-methylation is desirable but slow whilst the slower de-acetylation is undesirable but unavoidable to some extent.

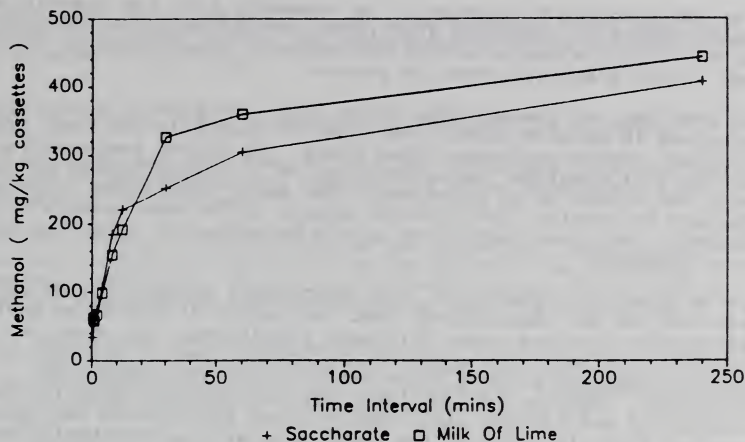
The factors which effectively control the extent of these reactions are considered to be as follows:

1. The reaction temperature. This is, in turn, dependent on the cossette temperature and that of the materials used for the treatment. The use of low temperatures, preferably 15°C or less is essential to prevent pectin breakdown (see below).

2. The penetration of the reagents into the beet tissue. With undamaged beet tissue, e.g. carefully prepared beet slices, it is possible to show that penetration of milk of lime is minimal beyond the first few layers of cells. McCready showed this with a lime slurry and also demonstrated that for cossettes made in the normal manner the situation was quite different with penetration through to the centre of the cossettes (Goodban et al., 1965). This is because the action of the knives causes extensive transverse cracking of the tissue, allowing access by reagents.

3. The nature of the reagents used. We have carried out most of our research and all of our factory trials with milk of lime whereas, recently, others have used saccharate solutions. With milk of lime most of the reactive material is in suspension and not in solution. It must, therefore, take a finite time for the majority of the reagent to become available as the soluble part is used up in reaction. With a saccharate solution, equivalent in calcium content to 1% CaO, all the reagent is in solution although the initial concentration of the calcium ion in solution is very similar in both situations.

Is there a difference, therefore, in reactivity between a saccharate solution and milk of lime? In terms of the de-methylation reaction we consider that there is little practical difference as shown by the release of methanol from cossettes treated with milk of lime or calcium monosaccharate solution illustrated in Graph 1.



Graph 1. Methanol release from alkali treated cossettes

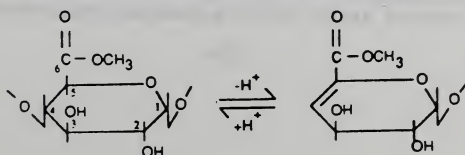
In the first 20 minutes during which the reagents, either calcium saccharate made from thin juice at 15°C plus finely ground calcium oxide and containing 1% CaO or factory milk of lime added to cossettes to give 0.4% CaO on beet, contacted the cossettes the rate of release of methanol was very similar in each case.

In laboratory diffusions of cossettes pre-treated with the two reagents we have not seen any clear differences in performance and each has given pulp which has pressed to a high dry substance in comparison with normal pulp.

iii. Cleavage of the glycosidic linkage

Pectin depolymerises relatively easily under alkaline conditions at high temperatures. The rate of depolymerisation, at any time, is proportional to the amount of methoxyl groups remaining, whilst pectic acid, with no residual methoxyl groups, is stable under alkaline conditions.

As early as 1955 Kenner (Towle et al., 1973) suggested that the mechanism involved was that of beta-elimination:



For the reaction to proceed it is necessary that the transition state should be resonance stabilised, requiring an acidic proton at C5. This is possible with the methoxyl substituent at C6 but not if the free acid group is present.

In practice, at temperatures below 20°C, the rate of glycosidic cleavage is so much slower than that of demethylation that insignificant pectin breakdown takes place. At higher temperatures the rate of cleavage increases relative to demethylation and at 70°C the effect is so pronounced that basic conditions, such as failure to acidify the diffusion supply water when ammonia is present, can cause sufficient pectin breakdown to interfere with pulp pressing.

It is essential, therefore, that sufficient de-methylation is achieved at low temperatures that the pectin is protected against glycosidic cleavage under diffusion conditions. In order to achieve this condition a period, no longer than 20 minutes, of intimate contact between cossettes and alkaline reagent is necessary. Shorter contact times are satisfactory both in the laboratory and factory situations but the explanation for this is not yet clear in terms of the known chemistry as demethylation will be incomplete.

Protein behaviour

Protein forms only a small fraction of the beet tissue but plays an important part in juice purification. During diffusion some of the protein fraction is solubilised and can be found in the raw juice. At high pH, and in the presence of calcium, this protein is precipitated from solution. The reaction is reversible to some extent and there is an optimum pH at which the maximum proportion of the protein is precipitated. Experience in those factories using prelimers, and in the laboratory, shows that the manner in which the optimum pH is approached also affects the resistance to re-solution, i.e. re-peptisation, of the precipitate and therefore careful control is necessary.

If cold cossettes are treated with an alkaline, calcium-rich, solution there will be no reaction with the protein of the intact cells, as they are relatively impervious to such solutions until the cell wall has been denatured, by the high temperatures of diffusion.

However, the process of slicing to form the cossettes causes severe damage to several layers of cells at the cossette surface. The cossettes therefore are covered in a layer of cell fluids which contain materials, including proteins, which can react with calcium-rich, alkaline, solutions. The reactions involved will be essentially those of juice purification and will produce flocculant precipitates containing calcium-bound protein and pectin with calcium salts such as oxalate and phosphate. The

presence of these materials, precipitated under less than ideal conditions, could lead to serious problems in the subsequent processing of the juice.

CHEMICAL OBJECTIVES OF ALKALINE PRE-TREATMENT OF COSSETTES

The key chemical reactions to consider are those of pectin and also those associated with the surface juice from the cells damaged during slicing.

From our present understanding of the scientific principles relevant to alkaline diffusion we can propose the following chemical objectives, which, if achievable in practice, should lead to a successful implementation of this new methodology;

1. De-methylation of the carboxyl groups of the polygalacturonate chain of pectin such that subsequent exposure to high pH does not cause cleavage of glycosidic linkages resulting in solubilisation of the pectin and loss of pulp structure.
2. De-methylation of those carboxyl sites which can usefully contribute to increased pulp rigidity as a result of calcium bridging. At present we have no means of achieving such selectivity and therefore it seems likely that such overall alkaline treatments as are being used will produce some free carboxyl sites which merely bind cations but cannot take part in the formation of bridges due to their steric situation. Exactly how much de-methylation is needed to achieve this, i.e. is it necessary to completely de-methylate or are some sites more critical than others, is at present unknown.
3. Incorporation of sufficient calcium ions to make maximum use of the bridging opportunities with minimum excess calcium bonding in non-bridging situations. This if achievable would give the best pulp pressing with minimum bound calcium in the end product for use as animal feed.
4. Minimum de-acetylation of the polygalacturonate chain. Release of acetic acid has no benefit, unless the reported decrease in sugar-end viscosities in the presence of acetate is relevant (Tiszavary et al., 1962), but has negative effect on pulp yield and juice purity. As the reaction rates of de-methylation and de-acetylation are similar there is, at present, no means of promoting one without the other.
5. Prevention of a rapid, step-like, increase of pH of the free surface juice present on freshly sliced cossettes, to avoid precipitation of juice components under non-optimum conditions. Two approaches are at present being followed; either the juice is removed by washing or the pH rise is arranged to occur gradually with the juice still present.

6. Control of the pH and the calcium concentration during diffusion so that there is minimum degradation of un-protected pectin and minimum formation of calcium-based precipitates in the diffusion juice.
7. Control of the ionic content of the diffusion supply water so that the bridge-forming calcium can remain in place in the exhausted pulp.

POTENTIAL BENEFITS

Based on our own laboratory work, as yet unconfirmed in our factory trials, and on Ponant's experience in France, as indicated below where appropriate, we consider that the most probable benefits from an alkaline diffusion process are as follows:

1. An increased pulp dry substance, following a normal pressing operation, reaching a maximum of about 42%. We have achieved this in the laboratory and Ponant has achieved it for a short time in a factory. Press modification may yield even better results but this is not yet tested.
2. An increased yield of pulp solids of at least 10%. We have laboratory data indicating an increase of 11.6% whilst Ponant has indicated 10% from trials in the 1989/90 campaign.
3. Reduced sugar losses due to acid and enzymic inversion in the diffuser, the magnitude of which is dependent on the current loss in the factory concerned. There is also the possibility of reduced bacterial activity but this is less certain in the absence of data from a long trial period. Some of the gypsum addition for pressing will no longer be required, reducing the sugar loss to molasses from incomplete removal of sulphate.
4. Reduced requirement for several process chemicals. Some calcium will probably need to be added to diffusion supply water to maintain good pressing but best present knowledge suggests that about 400 mg/litre gypsum will be sufficient i.e. about half current levels. No acid addition to diffusion will be required and biocide requirements may be reduced. There is a marked reduction in the amount of foam produced in diffusion which may eliminate the requirement for antifoams in process.
5. Ponant's factory studies have shown the possibility of increased press throughput, probably as a result of the combination of better draining before pressing and the inherently better pressing characteristics.

6. Ponant claims consistently reduced white pan viscosities with consequent reduction in pan boiling times.
7. Thick juice colours were reduced by more than half in Ponant's trials but this may not be reflected in white sugar colours.

Against these benefits we should note the potential disadvantage of an increased demand for soda ash in 2nd carbonation in order to maintain lime salts at conventional levels. We estimate that a 25% increase would suffice but that in practice none may be needed as scale production may not be a problem.

BRITISH SUGAR RESEARCH

Early research in British Sugar was along the lines suggested by Randall's work (Camirand et al., 1981) involving treatment of cossettes with calcium hydroxide and diffusion in a model Roberts battery. Other calcium compounds were tested and work with milk of lime became prominent because it gave equally as good results as other reagents and would be available in the factory.

A laboratory-scale continuous single screw DDS diffuser was obtained and comparison was made between its operation under the normal regime and that of the proposed alkaline diffusion. Some typical results from this type of work are shown in Tables 2 and 3.

Early experimental work with the model diffuser, using a manual method for mixing cossettes with milk and lime, showed that the resultant pulp and juice varied in composition and properties to a limited extent in response to the control parameters. The pulp almost always pressed to a high dry substance in comparison with that from a normal diffusion.

An experiment was therefore carried out to establish the relationships between the control variables and the output parameters. The apparatus used is shown in Diagram 4 and consists of a manual mixing system, a scalding rough, the model DDS diffuser and either a twin-screw continuous press or a batch hydraulic press. The following 12 control variables were used in a Plackett-Burmann experimental design together with the 16 output parameters:

Control variablesOutput parameters

% CaO in milk of lime
 % CaO in beet sliced
 Reaction time before scalding
 Milk of lime temperature
 Scalding temperature
 Diffusion temperature
 Diffusion time
 Sucrose in milk of lime
 % unreacted cossettes
 Calcium in DSW
 pH DSW
 Ammonia in DSW

Pressed pulp dry substance
 Wet pulp particle size
 Pectin in pulp
 Pectin in juice
 Protein in juice
 Calcium in pulp
 Calcium in juice
 Calcium in press water
 Wet pulp dry substance
 Press water pH
 Raw juice pH
 Sucrose in raw juice
 Invert sugar in raw juice
 Precipitate in raw juice
 % extraction
 Total energy of pulp

Note: DSW = Diffusion
 Supply Water

Table 2. Comparison of raw juice from treated and untreated cossettes

Raw Juice	Treated	Untreated
pH 20	11.5 - 12.5	5.9 - 6.4
Calcium (mg/l)	800 - 1500	33 - 130
Invert (g/100S)	0.26	0.54
Lactic acid (mg/l)	25	75
Acetate (mg/l)	1860	550
Phosphate (mg/l)	35	1078
PCA (mg/l)	203	60

Table 3. Comparison of dried unmolassed pulp from treated and untreated cossettes. Results shown as % on dry material.

Unmolassed pulp	Treated	Untreated
pH 20	7.5	6.0
Total crude fibre	20.3	17.4
Total crude protein	8.9	8.7
Total ash	13.6	4.5
Acid insoluble ash	1.3	2.0
Calcium	4.12	0.85
Magnesium	0.41	0.16
Phosphorus as P	0.69	0.12

Diagram 4. Laboratory apparatus for alkaline diffusion.

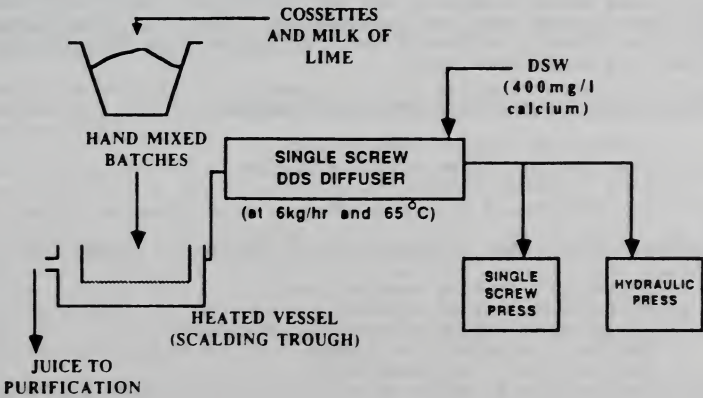


Table 4. Values of the control variables, in comparison with the normal target values, used in Plackett-Burman design.

Control variable	Low	Target	High
% CaO in milk of lime	8	10	12
% CaO on beet sliced	0.3	0.4	0.5
Reaction time (mins)	1	5	10
MOL temperature (°C)	10	20	25
Scalding temperature (°C)	70	80	90
Diffusion temperature (°C)	55	65	72
Diffusion time (mins)	100	120	140
% sucrose in MOL	0	10	12
% unreacted cossettes	0	10	15
Calcium in DSW (mg/l)	200	400	800
pH DSW	7	8	10
Ammonia in DSW (mg/l)	0	200	400

For each control variable two values were chosen, as shown in Table 4, usually to represent a high and low value, in comparison with our best understanding of what was the optimum target, such that we would expect some disturbance of the output parameters.

The experimental work was completed successfully with no major problems.

Of the 192 possible cause-effect relationships about 50 had statistical significance at the 95% confidence level and these in most cases agreed with our expectations. The inter-relationships which most closely relate to the anticipated benefits from the process are summarised below:

Pressed pulp dry substance was increased by:-

- increasing CaO% on beet sliced
- long reaction time before scalding
- high calcium in DSW
- low sucrose in milk of lime

Increased pulp pectin (increased pulp yield) was caused by:

- increasing CaO% on beet sliced
- high % CaO in milk of lime
- increased reaction time before scalding
- low sucrose in milk of lime
- high milk of lime temperature
- low ammonia in DSW

Reduced protein in juice (increased pulp yield) was caused by:

- lower CaO% on beet sliced
- increased reaction time before scalding
- lower scalding temperatures

Increased calcium in pulp (increased pulp yield) was caused by:

- increased CaO% on beet sliced
- increased reaction time before scalding
- high DSW pH

Increased total energy of pulp was caused by:

- lower CaO% on beet sliced

Other significant relationships included reduced invert sugar in raw juice as CaO% on beet increased and as scalding temperature increased. The amount of precipitate in juice, however, had no significant relationship with any of the control variables which was unexpected and disappointing.

Overall it is possible to summarise the results in terms of process control parameters as follows:

- CaO% of beet should be increased above target level (0.4% on beet) to test for potential increased benefits,
- reaction time before scalding should be increased until no further benefit is seen,
- maintain high calcium concentration, high pH but low ammonia in diffusion supply water,
- other parameters are already in good balance at present target levels and should remain fixed until in-factory optimisation is possible.

Once laboratory trials had shown encouraging results, confirmed by a visit to the Arcis-sur-Aube pilot plant of UCB, it was considered appropriate to move directly to factory trials. The alternative, of constructing a pilot plant, was rejected for two reasons. First, constructing a pilot plant would be costly, approaching the cost of full-scale trials; second there would be an unavoidable delay to progress of at least one and probably two or more years. In any event the scale up to full size would still be a large step to be overcome and much would be learnt from initial trials at full scale.

King's Lynn factory has a mean daily slice of about 5250 tonnes and operates one RT2 diffuser with a deSmet prescaler. Juice

purification is by the Dorr system, in use at all British Sugar factories, involving defeco-carbonation, clarification in Dorr thickeners and separation by rotary vacuum filters.

There have been four phases of development work at Kings Lynn factory.

Phase 1

Initial trials involved spraying undiluted, uncooled milk of lime (MOL) onto the bed of cossettes in the prescalder at a rate calculated to give 0.4% CaO on beet. The normal heat exchange function of the prescalder was inoperative during the trials.

The results were unsatisfactory particularly in terms of juice purification where settling and filtration became impossible within two hours.

The lessons learnt from this experiment were considered to be:

- i) although the overall heat balance of mixing cold cossettes with hot MOL to give 0.4% CaO on beet shows less than 4°C rise in cossette temperature, the effect at the surface of the cossettes may be the controlling factor. Before a temperature equilibrium can be reached the pectin of some of the outer cells could have reacted with the hot MOL, leading to glycosidic bond breakage. Evidence for this is mainly that of the slimy nature of the treated cossettes and comparison with laboratory simulation.
- ii) in order to contact the whole of the contents of the prescalder, which has a cossette bed depth of more than one metre, it would be necessary to over-dose with MOL to ensure penetration and then to pick up any excess MOL drained from the bed for re-cycle or discard. One consequence of this would be a tendency for the upper cossettes in the bed to be over-limed if the lower cossettes were correctly limed. To avoid this effect it was considered that some form of washing of the excess lime from the bed might be effective.

Phase 2

A plant for cooling factory MOL to a target temperature of 15°C was installed and provision made to saturate the bed of cossettes in the prescalder, collect the drainings and recycle them to the saturation stage. As MOL was removed from the system by absorption on the cossettes, more was added to the collection trays on a level control system.

A cold water wash, to remove excess unbound MOL was installed in the prescalder following the saturation stage.

In practice the results were very poor, it never being possible to control the amount of MOL picked up by the cossettes nor the amount remaining after washing. Although the target addition was still 0.4% on cossettes, values of from 0.2 to 3% were recorded.

The performance, as far as juice processing was concerned, was the same as the earlier trial.

The additional lessons learnt were:

- i) with cold MOL, whatever the amount added up to 3%, the cossettes were never slimy, indicating that MOL pre-cooling was an essential element in the scheme.
- ii) in order to make further progress it was considered essential to reach the target MOL addition rate without periods of over or under-addition, to avoid processing problems in juice purification.
- iii) some provision for handling a raw juice which was likely to give settling and filtration problems was required.

Phase 3

Research focussed on methods of achieving accurate target addition of MOL quickly and two approaches were considered, direct spraying onto cossettes and immersion of cossettes in a bath of MOL, suitably diluted, for a fixed duration.

The immersion process was laboratory tested and looked promising. Apparatus to carry out such a procedure in the factory was designed and costed but rejected at this time as the direct spraying approach offered the same performance, based on laboratory tests, at lower cost.

By direct spraying it is intended that each cossette would receive its own correct dose of MOL, from a spray jet and as little as possible by subsequent percolation from the mass of cossettes.

The only practicable way for a spray to contact all surfaces of a cossette is to drop the cossette freely through the spray. In the factory the cossettes fall from the slicers to the conveyor beneath and this region appeared to offer the most easily implementable option for spraying.

Spray nozzles, with or without air dispersion, were mounted beneath each of the six slicers to give as uniform as possible coverage of the falling cossettes. Flow rate of MOL to each nozzle was metered and the total flow rate ratioed to the slice rate.

Although the performance was not perfect it represented a significant step forward from earlier approaches and, at the target mean addition rate of 0.4% CaO on beet, the mean standard deviation was 0.06% CaO on beet in comparison with laboratory hand mixing sd. of 0.04.

Overall the factory results were unsatisfactory but revealed a potential weakness of the RT diffuser for alkaline diffusion. Cossettes treated with MOL are brittle in comparison with untreated cossettes and tend to break easily rather than bend when stressed. The exhausted pulp from the diffuser was always found to be broken such that the mean particle size reduced from 6cm normally to 2cm length under alkaline conditions and press performance was poor.

Juice processing became progressively worse during a typical trial, with unacceptable settling rate and rotary vacuum filter operation.

Lessons learnt from these trials included:

- i) there is a need to minimise the quantity of precipitate present in the raw juice. When this precipitate was removed by centrifugation in the laboratory the clear juice produced underwent either batch or continuous Dorr carbonation without problem. Return of the precipitate in increasing quantity gave all the symptoms seen in the factory purification stage.
- ii) although it might be relatively easy to tackle the removal of precipitate from the raw juice, the key to further progress would be the achievement of a satisfactory quality of pulp from the diffuser. In the laboratory DDS diffuser the extracted pulp retains its form, most of its rigidity and its particle size even when treated cossettes from the factory spray system are processed. Conditions within the factory diffuser must therefore be sufficiently more aggressive, either to make the whole operation unrealistic, or to make the initial cossette treatment much more critical than the laboratory work had indicated. (It should be added, however, that UCB (France) have achieved satisfactory experimental results, as far as diffusion and pressing were concerned, when using an RT diffuser in some of their early trial work.)
- iii) stoppages of the King's Lynn diffuser due to overloading were followed by a rapid fall in the pH of the contents. This has not been successfully reproduced in the model system but indicates a potential operational problem. The juice recovered after restarting the diffuser was exceedingly difficult to process, with adverse settling and filtration characteristics at carbonatation.

Phase 4

Ponant's operation, in France, also confirmed the importance of eliminating any precipitate from the raw juice. In his experience such precipitates arise as a result of two effects. First, surface juice from the cells ruptured during slicing will react with the high pH, calcium-rich environment to form a precipitate similar to that of juice purification. Second, any free CaO which enters the diffuser will be able to cause some further reaction there, perhaps involving the pectin material of the cell walls but also the diffusion juice, resulting in the formation of a precipitate.

Ponant avoids these effects to a great extent by removing the surface juice in his countercurrent mixer and by use of saccharate instead of MOL.

For our operations last campaign we did not have the facility to remove the surface juice before spraying, nor to use a saccharate solution prepared in the manner Ponant recommends.

Instead we tested the concept of removing any precipitated material formed as a result of liming, and any excess MOL, by washing in the prescalder with cooled 1st carbonation juice using about 25% on beet sliced. The washings from the prescalder were kept separate in a holding tank with the intention of allowing the process to reach equilibrium before they were returned to raw juice for processing.

Initially a single-pass washing was installed but this was quickly modified to a double-pass counter-current system when it failed to achieve the results predicted from laboratory work.

Even so, although it was possible to wash off all excess, unbound, CaO and any precipitate, the exhausted pulp was unsatisfactory, being badly broken as in previous trials.

Juice purification was again adversely affected, with filtration rate at first carbonation reduced by a factor of about five, although true equilibrium was not reached before the trial was stopped.

The overall conclusions from these factory trials are:

- i) although direct spraying appeared to give the most even and complete coverage of the cossettes with MOL, it did not lead to a satisfactory pulp pressing or juice purification.
- ii) it is unlikely that the performance of the spray system could be significantly improved by further development.

- iii) it is considered that the main factor affecting press performance is the breakage of the pulp during diffusion and that future work will be targeted at improving this aspect of the operations.
- iv) although it is relatively easy to saturate the free carboxyl groups of pectin with calcium ions, it is not easy to achieve significant de-esterification of the remaining carboxyl groups, in the time available, when using MOL in the factory situation. Thus the goal of maximum calcium uptake is not easily achievable.
- v) the problems experienced during juice purification, generally attributed to the use of MOL, are in principle solvable by removal of the precipitate which is formed, before purification. It is considered, however, that prevention is a better option and the use of saccharate solution should be tested.

OTHER EUROPEAN RESEARCH

In addition to British Sugar there are at least three other groups currently active in alkaline diffusion research.

Germany : The Braunschweig Institute, in collaboration with Warburg sugar factory, have carried out extensive studies on alkaline extraction using a small pilot plant. Buchholz and Schliephake reported this work following the 1988/89 campaign and have continued it during 1989/90 (Buchholz et al., 1989).

They have worked with calcium saccharate solution prepared from thin juice and milk of lime and have been particularly careful to prevent milk of lime entering the diffuser and to achieve a gradual increase of pH with time in the countercurrent mixer used for the cossette treatment. A DDS diffuser, operating at up to 30 kg cossettes per hour, was used for these studies and gave a raw juice which was processable and a pulp which pressed to 39.6% dry substance in comparison with a control experiment which gave 27.1% dry substance. The diffusion supply water was maintained at pH 9.5 and at a calcium hardness of 80 degrees.

All the predictable chemical effects were confirmed but juice purification was considered to be less than optimum because only incomplete preliming was possible as the raw juice was already at a high pH, up to 10.8, when it came from the diffuser.

Juice purification was by a classical system using an addition of 0.8% CaO at 85° Celsius, resulting in a final thin juice purity slightly below the control due to the presence of calcium acetate from pectin degradation.

In developing this work further it is considered that research into the possibility of suppressing the de-acetylation of the pectin and into the effects of acetate on sugar end operation, not all of which need be unfavorable (Tiszavary et al., 1962), will be needed.

During the 1989/90 campaign research was focussed on moving one step further by elimination of the diffusion process itself. Instead, following the alkaline pretreatment, the benefits of the improved dewatering characteristics of the cossettes have been utilised by direct pressing in two stages, with a small water return between the stages, and without any diffusion stage.

In the pilot plant the diffuser was used as a retention vessel to give the pretreated cossettes a residence time of at least 20 minutes before pressing.

The results, which are expected to be published in more detail later, showed an exhausted pulp dry substance of 50%, 98% raw juice on beet at 88.6% apparent purity. A yield of from 98 to 99% of the cossette sugar as raw juice sugar is claimed.

It is considered possible that development of this non-diffusion process to an industrial scale could proceed directly without first proving the diffusion process in a German factory. The process would give the benefits of fuel economy and the elimination of diffusion technology at the expense of some additional presses. It is considered that current press design is not, however, optimised for such work.

Italy: Accorsi and Zama have reported tests with a pilot plant using a saccharate solution prepared from thin juice, cooled to 20°C, and milk of lime (Accorsi et al., 1989). Operating at 300kg cossette throughput/hour, the plant consisted of an inclined mixing screw, a de-watering scroll and a continuous DDS-type scroll diffuser. Following diffusion, the exhausted pulp was pressed in a twin screw press, but the authors considered that this press did not reproduce the conditions experienced in factory presses and could only show the relative effects of different experimental conditions.

The data obtained from this experimental work confirm those obtained elsewhere in most details. Best results were obtained when the cossettes were treated with no more than 0.6% CaO on beet, greater than this causing marked rigidity in the cossettes and reduced extraction of sucrose. Even at 0.6% the cossettes were found to be more rigid than untreated cossettes and retained this rigidity through diffusion.

The exhausted pulp contained only about 7% dry solids and was pressed to give a dry substance of from 25 to 30% higher than pulp from a normal diffusion.

There was an increased yield of pulp solids, attributed mainly to the increase in inorganic ash content, as measurement of the organic marc did not show any difference from the control.

The raw juice from the diffuser was found to process normally in a laboratory purification based on a main liming scheme but without a preliming stage.

Unfortunately there is insufficient common ground between the pilot plant and their earlier, unsuccessful, work in a factory to extrapolate the results directly to the factory scale. The two main differences of significance are first, the factory extraction was by pressing followed by RT diffusion contrasted with the pilot scale DDS operation; second, the factory trial involved saccharate treatment at 60-65°C, allowing opportunity for β -elimination to occur, whilst this was considered to be absent at the temperature of the pilot work (20°C).

The authors see the major advantages of an alkaline treatment as the increased pressed pulp dry substance and its achievement without the addition of an anion, such as the sulphate contained in gypsum, thus reducing the sugar loss to molasses.

The benefits of less sucrose inversion and less diffuser corrosion are also mentioned but the underlying drive of the work appears to be the possibility of improving pressing before diffusion to such an extent that the diffuser is reduced to a much less costly item or is eliminated altogether.

It is likely that the research will continue towards an industrial process.

France: Ponant, working with UCB, has made the major reported progress in alkaline diffusion in the beet industry. Starting with laboratory work at the time of the oil crisis he has moved via factory trials, then a pilot plant at Arcis-sur-Aube to a full factory operation in the 1988/89 campaign at the Nangis factory.

In his early work he had experimented with the use of milk of lime but later developed the use of calcium monosaccharate solution which he patented in 1983 (Ponant, 1983). He considers that the use of a saccharate solution is essential because it is much less aggressive in action than solutions or suspensions of calcium oxide or hydroxide.

His process, which will be made available under licence, is shown in schematic form in Diagram 5. Calcium monosaccharate solution, which is free of undissolved calcium oxide, is made by reaction between fresh calcium oxide and a sugar solution, for example second carbonation juice.

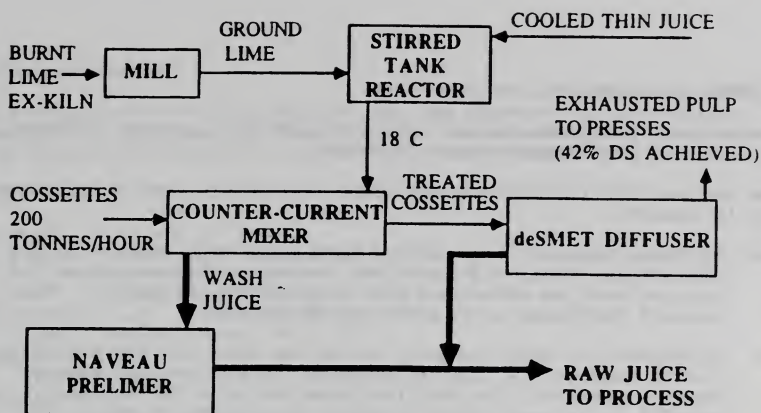


Diagram 5. Schematic of process in use at Nangis 1989/90.

The saccharate solution reacts with the freshly sliced cossettes in a countercurrent mixer, modelled on a DeSmet prescaler.

Spent saccharate liquor drains from the mixer and is treated separately from the main raw juice flow before a main liming and conventional purification.

The cossettes are diffused in a DeSmet diffuser at normal temperature but at an alkaline pH throughout.

The whole of the factory process follows closely the process described in his report of the Arcis pilot plant operations (Ponant et al., 1988).

The results obtained at Nangis are attributed to the major effort put in during their recent, but relatively short, campaign. The plant was operated for about 18 days with a continuous period of more than one week. During this time the sugar produced was of satisfactory quality and compared well with normal campaign production. Pulp production was increased, as was seen in the pilot trials, and dry substance was well above the normal value even though no press modifications were made.

From the results obtained it is most probable that the process will be quickly replicated within the UCB group of factories in France.

FUTURE DEVELOPMENTS AND PROSPECTS

No one has yet perfected an alkaline diffusion process although Ponant has come closest to this target.

We suggest the following areas where development may be expected or is needed:

- a) Diffuser type: at present the most successful operation has been with a DeSmet diffuser but to gain wide acceptance the process must be developed for tower diffusers and for "horizontal" diffusers of the DDS and RT designs.
- b) Purification: many European factories use the vigorous main liming schemes, an example of which is the process used at Nangis. However, for British Sugar to gain the maximum benefit it is most likely that we would wish to retain the modified Dorr system now in use. Development may be needed before the best results are obtained and therefore we will be targeting some of our efforts in this direction.
- c) Pressing: it is thought that a more freely draining pulp, coming to the presses at higher initial dry substance, may not be well suited, to conventional press design. In the first part of a twin screw press there is a section where water drains from the pulp before the compression of the material is intense. Less time may be needed for this process or it may not even be needed at all. Only experimental work with the new pulp material will allow optimisation of press design. Laboratory work with simple presses suggests that routine dry substance of about 42% may be obtainable and Ponant's recent work supports this view.
- d) Juice recycle: at present most workers are making the saccharate solutions for their trials from recycled thin juice. This in effect reduces the capacity or the retention time of the purification systems and increases the heat input needed in the raw juice heaters since the saccharate is made from cooled thin juice.
- e) Lime source: in the process currently proposed by Ponant the source of lime for the saccharate solution is fine ground lime fresh from the kiln. This procedure involves the installation of grinding mills which are likely to need significant maintenance and increase the initial expenditure.

Research is needed therefore to understand the criteria for effective saccharate production and to develop means of achieving this from lime either direct from the kiln or after minimal crushing.

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DISCUSSION

Question: Did you try enzymic treatments for the denaturation and deacetylation?

Okojie: No. Although other people are looking at enzymic treatments, we are not.

SAPONIN, A CAUSE OF FOAMING PROBLEMS IN BEET SUGAR PRODUCTION AND USE

Helena Hallanoro, Juha Ahvenainen, Leif Ramm-Schmidt
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INTRODUCTION

The foaming of sugar solutions in beet sugar production and use is a source of expense and processing problems. Saponins, being a surface active agent, have been considered to be a cause of foam and in acidic sugar solutions also a cause of floc problems.

In this paper the role of surface active agents in foaming is discussed. The extent of foaming problems and costs of anti-foam agents in the beet sugar industry is evaluated with the aid of an inquiry made among sugar companies in Europe and USA. Results of foam evaluation by two different methods of some white sugars are presented.

The occurrence in nature, properties and structures of saponins in general and especially beet saponins are discussed. The analytical methods used for the determination of saponin contents and composition are listed. The correlation of foaming and saponins is treated as well as the possibilities to decrease the saponin contents in the beet sugar process.

FOAM PROBLEMS

Foam, surface activity.

A foam consists of a gas dispersed in a liquid in a ratio such that the bulk density of the mixture approaches that of a gas rather than a liquid.

Kouloheris (1987) and Berger (1975/76) have explained that in a two-phase, gas/liquid, system foaming or defoaming takes place only because of the system's surface activity. Foams can not be produced from pure liquids - some type of surface activity must be induced to make the liquid foam. Surface activity is a change in the surface energy and the surface tension of the liquid, caused either by changes in the liquid's physical and chemical conditions or by the addition of surface active agent (surfactant) into the liquid. A surface-energy change may be an increase or a decrease in the surface tension of the liquid. Foaming or defoaming may take place depending on the net value of this increase or decrease, and the presence of a gas in the liquid. The phenomena of foaming and defoaming are indeed one and the same - both involving a change in surface tension. A

decrease in surface tension results in foaming; an increase in defoaming.

Surface active materials concentrate at an interface and the physical and chemical properties of the system are quite different at the surface compared to the bulk. This interface layer acts as a buffer, preventing the natural coalescence of the gas bubbles dispersed in the liquid.

Generally surfactants are composed of two portions, one with high affinity for solvent (hydrophilic when the solvent is water) and the other with no or little affinity to the solvent (hydrophobic). In order to meet the condition of minimum free energy required of all systems the surfactant molecules are either concentrated at the gas-liquid interface or they form in the bulk solution a cluster called a micelle. In a micelle the hydrophobic chains are in contact with each other and shielded from the polar water molecules. Surface activity is due to non-micellar surfactants, and the micelles act as a reservoir which increase the solubility of the surfactant, Fig. 1.

However, a reduction in surface tension alone is not enough to produce foam. The nature of the adsorbed layer is also important. Foam stability or foam elasticity, is what ultimately determines whether a foam is formed or destroyed. Usually, foam is stable if the surface tension of the solution is considerably less than that of the pure solvent. A stable foam also is one whose liquid film has a surface tension capable of enduring rapid, local variations in area. It is considered that this stabilization comes from one or more of the following types of compounds: surface active substances, macromolecules, particles of defined size and substances creating liquid crystalline phases.

When the gas in the solution is released, it has to be able to rise through the solution to the surface and low bulk viscosity is needed. If there is a low surface tension and a high surface viscosity the bubbles don't break when they reach the surface. Then a migration of surfactants by the concentration gradient formed takes place. A rise of the foam occurs if there is a high surfactant concentration over the critical micelle concentration, Fig. 2.

Defoamers and anti-foam agents.

Berger (1975/76) makes a distinction between defoamers, substances added to reduce existing foam, and anti-foam agents, substances added to prevent the occurrence of foam.

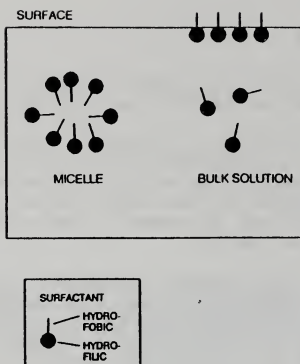


Figure 1.--Equilibrium system of surfactants

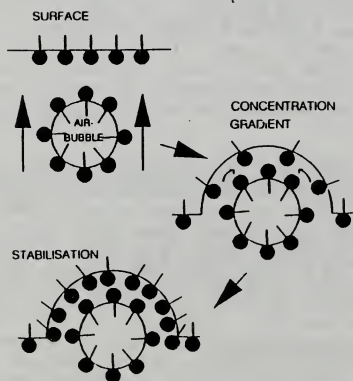


Figure 2.--Theory of foam formation

Anti-foam agents can act either as foam breakers or as foam preventers, and they can be considered as doing so by one of the following four mechanisms (Tjebbes, 1980):

- lowering of the surface viscosity, which can be achieved by large special molecules such as triborylphosphate
- creation of a concentration gradient as when spraying alcohol or ether on the foam
- changing the solubility of the foam maker by solvents or by altering the pH
- spreading of a film onto the foam lamellae

Commonly known anti-foaming agents are blends of various raw materials consisting of different chemicals such as fatty alcohols, special oxo-alcohols, fatty acid esters, fatty acid alkoxylates, paraffin oils, animal and vegetable oils as well as polyalkyleneglycolether (Potokar et al., 1982).

Foaming in the beet sugar process

Critical points of the process. In an inquiry made among sugar companies in Europe and USA the replying 13 companies informed about moderate (69%) or slight (31%) foam problems in the process. The critical process steps mentioned were:

- beet fluming and washing (in 62% of the answers)
- extraction, including prescalder (92%)
- juice purification (46%)
- evaporation (23%)
- sugar house, mainly after product (39%).

The juices and waters in beet sugar process contain several surface active agents and foam stabilizers of beet origin. Such compounds are e.g. fatty acids, probably as glycerides, lecithin, saponins, betaine, many amino acids and proteins. In the beet fluming and washing miscellaneous foaming material from soil is introduced with dirt particles as foam stabilizers (Tjebbes, 1980).

According to Tjebbes (1980) one must always remember that in most cases there is a mechanical cause for the foaming. It is thus always worthwhile considering whether or not the foam problem can be reduced simply by preventing leaks resulting in air suction at pump shafts, changing the resulting in air suction at pump shafts, changing the tank inlets or by other similar means. The shape and surface structure of the jets used in fluming are of fundamental importance for foaming in that area.

The beet sugar processing favours the foam formation through induction of gas to the liquids in several process steps: the cossettes bring air to the diffusion, carbon dioxide is led to the carbonatation and steam formation takes place in the evaporator station and the sugar house. Large amounts of foam are caused by the high velocities and the high impact forces in the flumes and by the beet washer.

The foaming causes many kinds of disadvantage:

- sugar losses through overfoaming of tanks
- decrease of capacity (pumps, diffusion, reaction tanks, etc.)
- costs of anti-foam agents
- inferior quality of the produced sugar (foaming, traces of anti-foam agents)

The use of anti-foam agents. The sugar beet processing companies spend 0.05-0.30 USD (average 0.14 USD) per tonne of beets processed for anti-foam agents. The majority of them has a centralized system for addition of anti-foam agents.

Tjebbes (1980) has presented a route of anti-foam agents through the process. Anti-foam agents were removed from the beet flume and wash water by the mud and must therefore be added continuously. Very little was adsorbed by the beet. Roughly equal proportions of the agents added into the extraction were found on the cossettes, in the carbonatation sludge and in the thin juice. Almost all of the anti-foam agent introduced into the sugar house, either by thick juice or by direct addition, ended up in the molasses. The concentration of anti-foam agents in the sugar crystals did not exceed 0.4 mg/kg.

An overdosage of anti-foam agents may cause additional foaming (Berger 1975/76).

By the use of the anti-foam agents there are some factors which has to be considered. The anti-foam agent

- must be safe for food use and during the handling for the factory workers
- must not cause any kind of harmful precipitates on thermal surfaces or elsewhere in the process equipment
- should not contain steam-distillable material more than 0.2% (Tjebbes, 1980) to avoid contamination of condensates to the power plant
- must be biodegradable
- should not have any negative influence on the biological treatment of waste water

Foaming in the use of beet sugar

About half of the sugar companies taking part in the inquiry has during the last five years had customer complaints from the confectionery and pharmaceutical industries due to the foaming. Complaints because of floc problems were as frequent as the foaming problems.

Foaming of sugar causes the biggest problems in the manufacturing of sweets in the open pan production. In this type of production the strongest foaming is usually found at the beginning of the boiling, when the temperature of the water-sugar-starch-sirup or water-sugar blend is 108-115°C. The foaming decreases as the boiling advances to the final temperature of 145-150°C and often ends up to a thin foam layer on the surface of the massecuite.

In connection with the foaming the customers have also complained about an increase in colour and an undesired crystallisation tendency. At the end of the boilings where foaming appears an exceptional cloudiness of the surface - a kind of "surface film" - may sometimes be noticed.

The above mentioned problems concern mainly the small producers using traditional boiling methods. In the continuous processes where boilings are carried out in either over or under pressure the problems are more difficult to observe because of the construction of the equipment. The sugar content of the massecuites often is lower than in the traditional open pan boilings. The surface level control is often performed with the aid of a capacitive measurement probe which can be misled by the foam with the consequence of disturbance in the control of the continuous process.

In some cases the use of anti-foam agents is not possible, which emphasizes the importance of a non-foaming quality of the sugar.

Evaluation of foamability

In the 17th Session of ICUMSA (1978) in Montreal (Subject 19) there was stated: "From time to time Recommendations have been made in earlier Reports on subject 19 or its precursors to the need for determining the foaming tendencies of white sugars, the "conditioning" status of white sugars, sucrose in white sugars and "alcoholic haze tendency". We believe that at least the first two of these are of substantial commercial importance and that a need for standardized and reproducible methods still exists. Perhaps in these instances there is not so much a need to study existing methods as to develop new ones; we recommend accordingly. Recommendations: 8. Methods should be sought for determining the foaming tendency and "conditions status" of white sugar." There are no remarks on this subject in the Proceedings of the 18th and 19th Sessions of ICUMSA.

In Table 1 there are details of different methods used in order to evaluate the foaming tendency of white sugar. The two main types are "the boiling methods" and "the gassing methods." The method according to Paine et al., (1924) or its modifications are perhaps the most commonly used. Problems with repeatability, reproducibility and sensitiveness may arise especially with the boiling methods. An other matter of concern is the validity of the results - how well they actually can describe the situation by the user.

Table 1.--Methods to evaluate the foaming tendency

Type of method	Sugar solution	Way of foam making	Measured values
Paine et al., (1924)	67 % w/w	boiling	Foam height or volume at first and 118°C boiling points
Paine, mod.	67 % w/v	boiling	Max. foam volume volume of solution
"Candy", 1	83 % w/w	boiling	Max. foam height
"Candy", 2	87 % w/w	boiling	Max. foam height in % of the height of the solution
Bikerman	40 % w/w	air suction 125 mmH ₂ O = 9 mmHg	Max. constant height of foam
Air gassing	40 % w/v	air gassing 50 mmHg 1750 ml/min	Foam height after 1 min
Symes et al., (1982)	60 % w/w 30 % w/w	N ₂ gassing 1200 ml/min	Foam height after 45 sec., collapse rate
Szekrenyesy et al., (1989)	process juices	N ₂ or CO ₂ gassing various rates	Max constant height of foam, relative

Foaming tendency of different white sugars

The foaming tendencies of different white sugars randomly collected from Europe, USA and Japan were evaluated by the methods of Paine and Bikerman. Details of the methods are in the Appendix. The results are presented in Figures 3(Bikerman), 4 (Paine) and 5 (Paine).

At Finnsugar we have regarded as "the safety limit of foaming" for the first boiling point 70 ml and for the 118°C boiling point 40 ml with the method according to Paine. Among the sugars evaluated 21% exceeded the first boiling point safety limit and even 40% the 118°C boiling point respectively. The result indicates the possibility of foaming problems by sensitive sugar users. We have not estimated the safety limits for the Bikerman method.

The dependence of foaming values at the first and 118°C boiling points is not very close as can be seen in Figure 6. Figure 7 shows the Paine boiling point values as a function of Bikerman-heights. The correlation is poor between these values. The average deviation between duplicate samples in these tests was about 10% in the Paine method and 5% in the Bikerman method.

Devillers et al., (1968) have published some foam tests values of white sugars between 1 and 9 cm made by the Bikerman method.

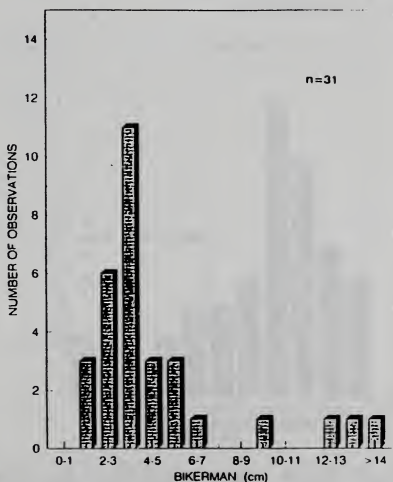


Figure 3.--Foam test of white sugar according to Bikerman

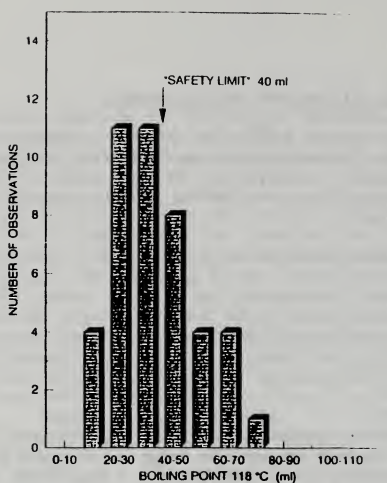


Figure 4.--Foam test of white sugar according to Payne, first boiling point, n = 43

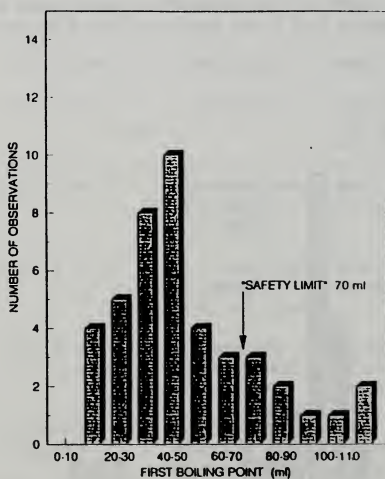


Figure 5.--Foam test of white sugar according to Paine, 118°C boiling point, n = 43

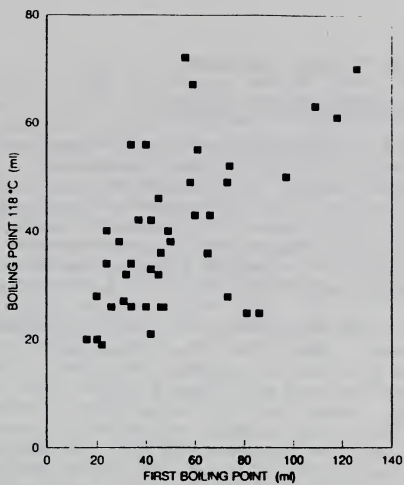


Figure 6.--Foam test of white sugar according to Paine, correlation of boiling point values, $n = 43$

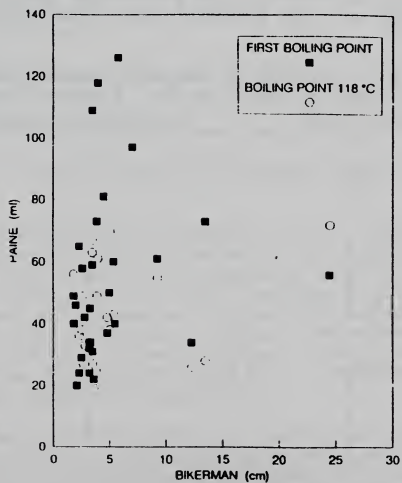


Figure 7.--Foam test of white sugar, correlation Paine vs. Bikerman, $n = 31$

SAPONINS AS A CAUSE OF FOAMING PROBLEMS

Saponins and their distribution in nature

The general properties of saponins have been described by Shibata (1977) and Trease et al., (1978). Plant materials containing saponins have long been used in many parts of the world due to their detergent properties, e.g. in Europe the root of *Saponaria officinalis* (Fam. Caryophyllaceae) and in South America the Bark of *Quillaia saponaria* (Fam. Rosaceae). A great number of species of saponins are distributed in higher plants, while some marine animals, such as seaslug and starfish, also produce saponins.

Such plants contain a high percentage of the glycosides known as saponins (L. sapo, soap), which are characterized by their property of producing a frothing aqueous solution. They also have haemolytic properties and are highly toxic when injected into the blood stream. Saponins have a poisonous effect to fishes and shells. When taken orally saponins are comparatively harmless. Sarsaparilla, for example, is rich in saponins but has been widely used in the preparation of non-alcoholic beverages. Formation of precipitates with cholesterol in alcohols as well as antimicrobial activities, especially antifungal activities, have been demonstrated as characteristic properties of saponins. The crude drugs which contain saponins are generally used because of their detergent properties, but saponins have also other pharmacological applications.

The dependence of surface tension on saponin concentration in water solution is published by Joos et al., (1967). Figure 8 is based on this data.

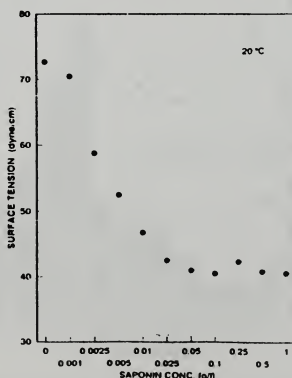


Figure 8.--Surface tension of saponin solutions.

Saponins have a high molecular weight and their isolation in a state of purity presents some difficulties. As glycosides they are hydrolysed by acids to give an aglycone (sapogenin) and various sugars and related uronic acids. According to the structure of the aglycone or sapogenin two kinds of saponin are recognized, the steroidal and triterpenoid types, Figure 9. Both of these have a glycosidal linkage at C-3 and have a common biogenetic origin via mevalonic acid and isoprenoid units. The structures of numerous sapogenins have been established, whereas until the 1960s only a few saponins were chemically described. This might be caused by the difficulties in purification of saponins, which, however, has been improved by the remarkable developments of various chromatographical techniques.

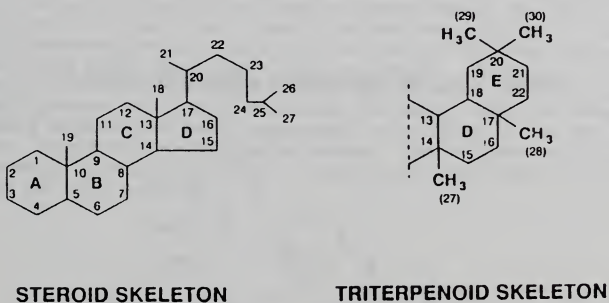


Figure 9.--Two aglycone types of saponin

By the investigation of Guvonov et al., (1970) on 1730 species of 104 families of plants growing in the Central Asia, triterpenoid saponins were found in 627 species, and steroidal saponins in 127 species. About 76% of plant families so far examined contained some amounts of saponins. This would suggest a wide distribution of saponins in the plant kingdom. Tschesche and Wulff (1973) referred in their review entitled "Chemie und Biologie der Saponine" to 43 steroidal and 110 triterpenoid plant saponins and established chemical structures.

Most triterpenoid saponins are pentacyclic and the sapogenin is attached to a chain of sugar or uronic acid units, or both. Biosynthesis involves cyclization of squalene. Triterpenoid saponins may be classified into three groups presented by α -amyrin, β -amyrin and lupeol. The related triterpenoid acids are formed from these by replacement of a methyl group by a carboxyl in position 4, 17 or 20.

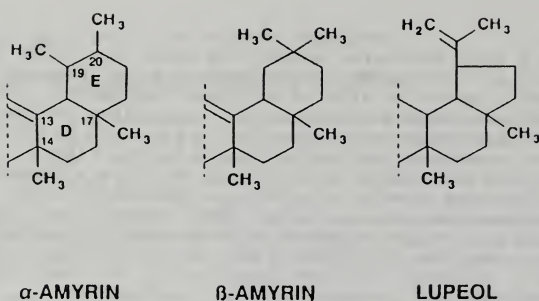


Figure 10.--Triterpenoid sapogenin types

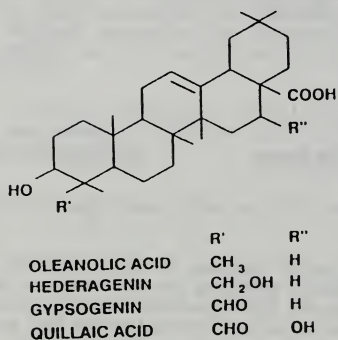


Figure 11.--Typical triterpenoid saponins

Plant materials often contain triterpenoid saponins in considerable amounts. The primula root contains about 5-10%; liquorice root about 2-12% of glycyrrhizic acid; quillaia bark up to about 10% of the mixture known as "commercial saponin"; the seeds of horse-chestnut up to 13% of aescine. In the Figure 11 there are some typical sapogenins of the triterpenoid saponins.

Sugar beet saponin

The sugar beet saponin has been a subject for investigations already over one hundred years. At the beginning glycosidic compounds were isolated from sugar beet cells, carbonatation sludge and factory foams and they were called cholesterin, isocholesterol and phytosterol. After hydrolysis of the glycosidic compounds the so-called beet resin acid was obtained. In 1911 Smolenski deduced the other part of the glycosidic compound to be the glucuronic acid. In 1927 van der Haar isolated three saponins with different solubility properties and recognized the beet-resin acid to be oleanolic acid. Eis et al., (1952) found three compounds as parts of beet saponin: oleanolic acid as free aglycone, the glucoside composed of oleanolic acid and glucuronic acid (Figure 12) and a glycoside-salt complex giving glucuronic and oleanolic acids on hydrolysis. Ten years later van Duuren (1962) isolated with thin layer chromatography six different sapogenins in sugar beet saponin which were probably closely related substances of the β -amyrin group. Besides oleanolic acid one of them might have been quillaic acid. Shiga et al., (1964) compared the infra-red absorption spectrum of purified beet saponin powder with that of oleanolic acid, and the characteristic wave numbers of both compounds were found to be the same. Using a paper chromatographic method, the authors confirmed the presence of glucuronic acid, glucose and arabinose in the hydrolyzate of beet saponin.

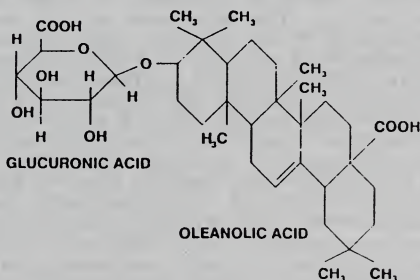


Figure 12.--Sugar beet saponin

The saponin content of sugar beets has been investigated by several authors: Shiga et al., (1964), Wagner and Sternkopf (1958), Lüdecke and Feyerabend (1958), Winner and Feyerabend (1971) and Rother (1962). The contents announced show a large variation between 0.01 - 0.2% on fresh beet. The reason for

deviations in these values may be found in the used methods of isolation and purification. The methods of analysis and quality of standards used are not uniform. E.g. the yield of the purified saponin powder on the crude powder has been only 2.0-7.1% (Shiga et al., 1964). The saponin content of beet peel was five times higher than that of the central part, and the lower part of the root contained 60% more than upper and middle parts (Shiga et al., 1964).

The unbroken beet skin is an effective barrier against the invasion of microorganisms (Halden, 1982). This action is probably aided by the high concentration of saponins located just under the skin.

The beet saponin has haemolytic activity which has been quantified by Shiga et al., (1964) and Wagner and Sternkopf (1958). Nagornaya et al., (1966) mention the inflammatory effect of beet saponin to mucous membrane, toxicity to fishes and its possible risk in the use of molasses as animal feed. However, Wagner and Sternkopf proved with rat experiments that beet saponin is in defined limits a growth stimulant due to its surface active properties and non-toxic for sound animal organism, since it was not be resorbed.

The properties of sugar beet saponins as published in the literature (Eis et al., 1952; Rother, 1962; Nagornaya et al., 1966; Wagner and Sternkopf, 1958) have different values. This may be due to the different compounds included into the beet saponin and also its varying purity. The appearance of the isolated saponin powder is mainly almost white, perhaps light yellow, grey or tan-coloured. Beet saponin is soluble in NaOH, KOH and NH₃ water solutions, glacial acetic acid and in lower alcohols. It is insoluble in chloroform, benzene and acidic water solutions. Most contradictory is the data concerning the ether solubility. There are authors who have isolated ether insoluble saponin and others who have got soluble saponin. However, Eis et al., (1952) have showed that the glycosidic salt complex part of saponin isolated from factory diffusion juice is ether insoluble whereas the ether soluble parts contain the free aglycone oleanolic acid and glycosidic oleanolic-glucuronic acid compound. McGinnis (1972) reports that when solution in alkali and precipitation in acid is repeated a number of times, the saponin becomes more and more insoluble, until eventually it can hardly be redissolved at all.

The values of melting points differ due to the same reason as the solubilities (Eis et al., 1952; Nagornaya et al., 1966; Rother et al., 1962; Shiga, et al., 1964; Wagner and Sternkopf 1958). The most common value given is 214 - 216°C which corresponds to the glycosidic oleanolic-glucuronic acid compound. Unpure powders

give lower values down to 190 - 200°C and oleanolic acid alone 296 - 306°C depending on the purity.

The beet saponin is optically active (Nagornaya et al., 1966; Shiga et al., 1964), hygroscopic and can be precipitated with heavy metals (Eis et al., 1952).

Beet saponins have drawn the most attention because of their floc forming properties in acidic sugar solutions. These sugar quality problems are not only due to saponins which, however, seem to be the decisive factor. Other components of the floc are e.g. fats, traces of defoaming oils and other acid-insoluble compounds. (Shiga et al., 1964; Sabine, 1952; McGinnis, 1972 and 1984; Schiweck, 1963; Rother, 1962; Eis et al., 1952; Roberts et al., 1974 and 1976).

Analytical methods

The analytical methods used for determination of saponins derived from sugar beets or other plants present a wide selection based on the different properties of the saponins. A summary of the methods is presented in Table 2. Only one of the 13 companies in our inquiry tests the saponin contents then by the antimony pentachloride method. Another company has made the tests more unregularly by the same method. Some companies refer to visual floc tests as a measure of saponins.

Except the chromatographic methods the other methods of analysis are not specific to saponin compounds. Some of them are very time-consuming and laborious. There has been criticisms against the reproducibility and recovery of the methods (Rother 1962). The determination of triterpenes with antimony pentachloride seems to be quite accurate but recovery of saponins is only in order of 70% (van der Poel et al., 1964). Some sugar companies are at present developing HPLC- or GC-methods for more specific determinations of beet saponins. Corresponding chromatographic methods for saponins derived from other plants have been published as indicated in Table 2.

The commercial saponin standards are isolated from other plants than sugar beets. Hence their saponin composition is different compared with sugar beet saponins. The isolation and purification of sugar beet saponin standards has been described by several authors with some differences in the procedures: Shiga et al., (1964); Nagornaya et al., (1966); Walker, (1956); Rother, (1962); West and Gaddie, (1956); Johnson, (1974).

Table 2.--Analytical methods for determination of saponins

Method	Reference
Hemolysis test	Eis et al., (1952) Luedecke and Feyerabend (1958)
Fungistatic test	Biacs et al., (1982)
Gravimetric	Nagornaya et al., (1966) Eis et al., (1952)
Polarographic	Hibbert et al., (1961) Schiweck (1963)
Coagulation with quarternary amines (Hyamine)	Johnson & Diehl (1956) Hörning (1957) Rother (1962)
Colorimetric methods Antimony pentachloride	Walker (1956) West and Gaddie (1956) Hanzas and Barr (1969) Rother (1962) Hörning (1957)
Sulfuric acid	Nagornaya et al., (1966)
α -naphtol-sulfuric acid	Bauserman and Hanzas (1957a)
Liebermann-Burchard (glacial acetic + sulfuric acid)	Johnson (1974)
Tollens naftoresorcinol (uronic acids)	Eis et al., (1952)
Electroforesis + fluorescence	van Duuren (1962) Wagner and Sternkopf (1958)
Chromatographic methods	
Paper	Bauserman and Hanzas (1957b)
TLC	van Duuren (1961)
HPLC	Ireland et al., (1986) Burnouf-Radosewitch et al., (1986) Petersen and Palmqvist (1990)
GC	Burnouf-Radosewitch et al., (1985)

The connection between foaming and saponins

Paine et al., (1924) considered the foaming of sugar solution to be due to a combination of two factors: a formation of air-in-liquid dispersion and an hydrophilic depression of the surface tension. The stabilisation of the foam is thought to be of a colloid origin. In a massecuite of crystal sugar the air is adsorbed at the crystal surface, entrapped between the crystals and dissolved in the liquid. The depression of surface tension is caused by the trace constituents of the sugar - saponins, proteins, defoaming agents, inorganic compounds etc. Microbiological activity and quality of the water used may also influence foam forming. The stabilisation of the foam is dependent on the synergetic effects of the same trace constituents as the surface activity.

van der Poel et al., (1964) have studied the role of saponin in beet sugar foaming. They have determined the saponins with the colorimetric antimony pentachloride method and compared the results with foam tests according to Paine et al., (1924). White sugar with no saponins did not foam. They also found that sugar with less than 0.2 ppm saponins never foams and sugar with more than 0.4 ppm always foams, Figure 13. Also other foam forming components were studied by adding a number of substances to non-foaming white sugar. The protein products (gelatin and albumen), cholesterol (structure related to the steroid saponins) and anti-foam agent promoted foaming whereas the polysaccharides (dextran and agar agar) did not foam. The improper use of anti-foam agents which might leave traces of these compounds into the crystals can make the situation even worse. The saponins from raw juice gave rise to heavy foaming already at saponin contents of some ppm. The saponins from molasses didn't have so intense foam effects. The composition of the saponins in white sugar was also somewhat different from those in the juices so that sapogenin alone was present or at least in a greater concentration than the other saponins.

On the other hand, Oldfield and Dutton (1967) made experiments to identify the components mainly responsible for the foaming observed in some beet white sugars. They found saponins not to be major contributor to foam due to their low concentration in white sugar. A foaming sugar was made non-foaming by treatment with powdered carbon and a crude extract was obtained by eluting the spent carbon with hot acetic acid. This extract contained no

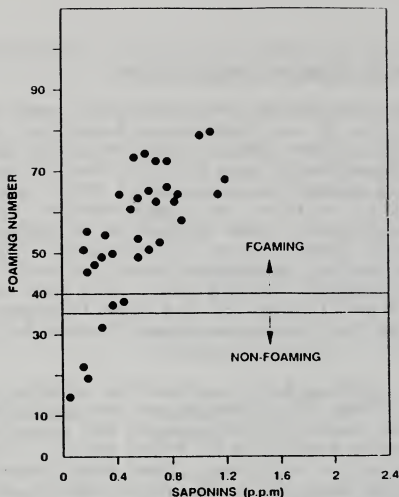


Figure 13.--Correlation between foaming of sugar and saponins according to van der Poel et al., (1964).

saponin, but did contain high molecular weight peptides which were found to induce foaming. The extract also contained a low molecular weight foaming component, which remained unidentified. In the discussion following the presentation of the Paper in the CITS Conference it was notified that the saponins may have been adsorbed by the carbon but were not eluted by the acetic acid into the foaming extract. So the actual role of the saponins in foaming still stayed somewhat obscure.

Devillers et al., (1968) have also published data connecting the foaming tendency of some sugars by the Bikerman method and saponin contents according to Walker (1956). The correlation between foam and saponins in these few sugars is clearly positive.

Symes and Vane (1982) concluded that the formation and stability of foam, for a given beet raw sugar, is strongly dependent upon concentration and temperature; it is highly probable that surface viscosities is an important parameter. A sample of protein studied proved to be a strong foam promoter, whilst not being very strongly surface active. The surface active impurities in the raw sugars studied appeared to be present in very small amounts, and are therefore of high specific surface activity. The surface tension results were consistent with saponin being a prime cause of the surface activity of the raw sugar studied.

Our preliminary studies with colorimetric methods developed by Nagornaya et al., (1966) and Bauserman and Hanzas (1957) didn't show any positive correlation between foaming properties and saponin concentrations. The poor reproducibility and recovery by the use of the methods may also have had an effect on the results.

Possibilities to decrease the saponin contents

The sugar beet. Lüedcke and Feyerabend (1958) have studied the saponin contents of the sugar beet in relation to the use of fertilizers, varieties and climate. They found out that the saponin formation has close relationship to the sugar formation and the contents of these both run almost parallel during the growing season of the plant. The high use of nitrogen fertilizer decreased the contents of sugar and saponin respectively, the level of potassium and phosphorous fertilizer had no influence. The Z-types which are varieties with high sugar content also had higher contents of saponin than the E- and N-types with lower sugar content. The effect of climate on the saponin formation could not be established although it could be noticed that favourable climatic circumstances gave higher sugar and saponin contents.

According to Eronen (1990) the biological activity of saponins in plants is still partly unknown. The saponins are situated in the membranes of the cell and they are evidently also a component of cell membranes: plasmalemma, tonoplast and plastids of the cytoplasm. They belong to the secondary compounds and they may be final products of the metabolism. Secondary compounds have an essential role in the plants ability to pass the winter and tolerate the coldness. When the beet begins to store sugar for the winter it also metabolizes saponin. The climatic factors delaying the maturation keep the saponin contents low. A too high use of nitrogen fertilizers delays the maturation. The plant stays in a more vegetative state and sugar and saponin contents are low. The beet varieties with high sugar content have smaller roots and cells and thus more membrane structures and saponin and vice versa.

It seems to be inevitable that there is no traditional way to reduce the saponin content of sugar beets without affecting the desired sugar content. With the aid of gene technology it might be possible to induce changes in the molecular proportions of the cell and thus produce a beet with high sugar but low saponin content.

The beet sugar process. As the highest saponin contents are found in the peel of the beet the damaged beets increase saponin contents in flume and wash waters. The liming to high pH favours the extraction and dissolving of saponin components. A decantation system which is operating well will reduce foaming

considerably as will the use of a flocculant (Tjebbes, 1980). The foam formation is hindered by the addition of lime at pH values higher than 10.5 as well as by using suitable anti-foam products (Bertuzzi, 1989).

Eis et al., (1952) studied the behaviour of saponins in the diffusion and found that triterpenes are more rapidly extracted than sucrose. They also concluded that the saponin concentration in raw juice can not be affected by reasonable changes in pH, draft, time or temperature. However, the saponin contents of the raw juice can vary widely from factory to factory. Hanzas and Kohn (1961) have given values ranged from 1660 ppm to 3670 ppm on DS and McGinnis (1982) 5000-10000 ppm on DS. Hanzas and Kohn suggest that this variation may be due partly to both location and variety of beets.

McGinnis (1972) has presented a gravimetrically determined floc (saponin) balance over the beet sugar process in the Woodland factory. In this factory 61% of the saponins in beet were extracted into the raw juice. The author supposes that if the pH of the supply water had been less alkaline (pH value not mentioned), probably much less saponins would have been extracted. The floc also seemed to be more easily extracted from degraded beets. Johnson (1960) has found in a laboratory trial that the extraction of saponin from beets increased when the supply water was in the alkaline range and especially when it contained ammonia. A lower saponin concentration in raw juice of 118 ppm analysed by the Walker method has been given by van der Poel et al., (1964).

When the beets were peeled with 8.9% loss on the original weight Edwards et al., (1989) could in a pilot plant experiment decrease the saponin contents of raw juice from 9100 ppm on DS (unpeeled beets) down to 7100 ppm.

In juice purification saponins are precipitated by calcium. 90 - 97% of the saponins in the raw juice are eliminated mainly in the first carbonatation (van der Poel, 1964; McGinnis 1972 and 1982; Hanzas and Kohn, 1961). van der Poel et al., (1964) showed that there is less saponins in the thin juice if more lime is used and if the end point of the first carbonatation is higher. The addition of lime up to approximately 0.15 - 0.25% CaO on beets at the second carbonatation resulted only in a minor reduction of saponin in the thin juice (Johnson, 1960) and no decrease in white sugar floc (Gaddie, 1956). However, McGinnis (1982) mentions that addition of a small amount of lime to the second carbonatation also helps in floc elimination. van der Poel (1964) states that there is a rather big difference from factory to factory and from year to year in the saponin contents of thick

juice. Thus the saponins content is yet another thing which depends strongly on the quality of the beet. No fixed rule was found that the saponin content will decrease or increase during the campaign.

The precipitation of saponins with heavy metals other than calcium doesn't seem possible because of the toxicity or higher solubility of the other metals in question (Eis et al., 1952).

In general good regular sugar crystals with as few conglomerates and inclusions as possible are a way to minimize the content of non-sugars in the white sugar. However, in the case of saponins the situation is somewhat different. Carruthers et al., (1961) demonstrated that the foaming and floc problems do not arise simply from the presence of a film of mother liquor. The addition of 0.2% molasses to a very high quality sugar increased the ash content by 0.02% but the molasses addition had no perceptible effect on the foaming index or on the floc precipitation characteristics of the sugar. In white sugar the measured saponin/potassium ratio was 8 times as high as in the corresponding massecuite. Verhaart et al., (1967) have shown with laboratory experiments and in factory practice that the removal of ash compounds is easier than the removal of saponins and colour substances. There seems to be a selectivity for the adsorption of saponins in the crystals. This is not very clear in sugars, which have been centrifuged without the use of wash water. The selectivity increases with increasing amounts of wash water. In sugars, which have been washed excessively in the centrifugals the ratio saponins/potassium is about 5 times as high as the same ratio on the corresponding massecuite. The elimination factor of saponins was determined to 98.9 in white sugars and 90 in intermediates when the e.g. for potassium is 99.6 and 94 respectively (van der Poel et al., 1964).

Devillers (1967) has reported that the effect of saponins on the rate of crystallisation is negligible at pH 8.1. However, at pH 6.1 saponins decrease the rate probably due to partial precipitation and adsorption on the crystal surface. Many authors McGinnis, 1972; Hanzas and Kohn, 1961; Johnson, 1960; Gaddie and West, 1958) have in accordance to this recommended crystallisation at steady pH values higher than 8.5 in order to keep the saponins soluble and the white sugar saponin content sufficiently low.

The only ways to be certain that the sugar produced is in safe limits of foaming and floc are sufficiently low saponin content and high pH of massecuites. A safe value for the former is < 10 ppm saponins (van der Poel et al., 1964).

If the desired low saponin content seems to be hard to meet with normal processing measures the white sugar might be recrystallized, but also then the selectivity for the adsorption of

saponins in the sugar crystals makes the result inferior. The treatment with adsorbing agents such as activated carbon or with ion exchangers has given juices with substantially lower saponin contents (Eis et al., 1952; van der Poel et al., 1964). On the other hand, Gaddie (1956) did not reach the required effect with activated carbon at the factory trial. These methods may present a substantial cost factor.

CONCLUSIONS

The sugar beet processing companies and sometimes also their customers have substantial problems due to the foaming in the process. Saponins as surface active agents are one of the foam causing factors and a reason for floc formation in acidic sugar solutions.

The amount of saponins decreases throughout the purification process of sugar and this decrease is dependant on the process steps involved. The quality of sugar beet is, however, the most important factor in determining the saponin content of white sugar. The aim of plant breeding and growing is to get beets with very high sugar content. This is likely to bring the disadvantage of increasing also the saponin content in beets.

In order to meet the sugar quality requirements of the customers a reliable and valid foam test should be developed. The work with chromatographic saponin determinations is expected to give a more specific tool in solving the foam problems.

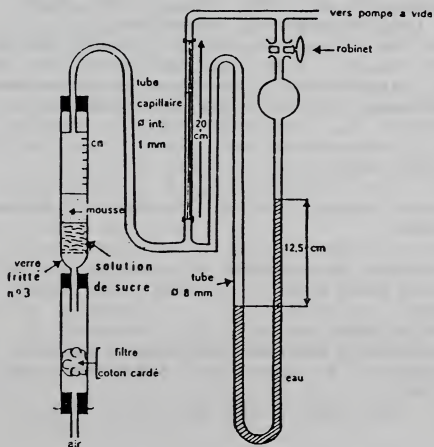
APPENDIX

Foam test according to Paine et al., (1924)

To 50 grams of sugar in a 200-cc. beaker, tall type, 25 cc. of water is added and the solution is heated gradually at a uniform rate to 117.8°C (244°F). The time periods required to reach the first boiling point and 117.8°C should be 3.5 to 4 minutes and 8 to 9 minutes, respectively. After dissolution of the sugar the beaker is marked at the liquid level and upon reaching the boiling point when foaming commenced a mark is made on the beaker at the level of maximum foaming. The data obtained are expressed in terms of volume.

Foam test according to Bikerman (Methods d'analyse I.R.I.S. 1984)

The height of the produced foam is measured in an apparatus of normalized dimensions, when air is passed through a sugar solution. The apparatus is described in the figure below. A glass frit (no.3) is placed at the bottom of the glass cylinder, whose diameter is 30 mm and height 300 mm. The cylinder is connected to a vacuum pump in order to keep the pressure constant (125 mm water). The test is performed at room temperature. 50 ml of sugar solution (40% w/w) is placed into the cylinder and height of the solution is measured. The pump is switched on and the height of the foaming solution is measured every 15 sec. until it has reached a constant value. The difference between original and constant foam heights is the result of the foam test.



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DISCUSSION

Question: You mention that the peel has the most saponin. What is your definition of the peel?

Hallanoro: It is approximately 1 millimeter.

Question: As much as 1 millimeter?

Hallanoro: Yes, in our definition.

Question: Thank you for the very comprehensive study and for the summary you presented on this problem. Did you look at the influence of the top and leaf material which comes in with the beet to our process more than it used to, due to changes in harvesting techniques. I am not quite sure about your conclusion that the beet quality is the predominant factor. I think that juice purification is more important. You can eliminate between 90% and 97%, which means that the rest of the saponin that goes into the thick juice varies between 10% and 3%, which is more than a factor of 3. In fact, juice purification is a very powerful way to reduce saponin content. I think that, together with pH control in the vacuum pan station, makes the problem manageable.

One of the advantages of alkaline extraction was mentioned as the lowered foaming ability of the juice. I agree with you that alkaline extraction will increase the saponin extraction. What is your opinion about that?

Hallanoro: We have not dealt with the influence of tops and leaf material. In alkaline diffusion, I think the saponins are more soluble; however, they might be precipitated as calcium salts.

Question: On Figure 4, foam test of white sugar, you determined foaming of forty (40) white sugars from different countries. Were these only beet sugars or was cane sugar included?

Hallanoro: Only beet.

Question: Is this foaming a problem only with beet sugars, or with cane sugars also?

Hallanoro: We have not examined cane sugars, but I think that beet sugars have a greater problem.

Question: About 30 years ago, when Coca Cola increased its market in Europe, we were told that floc problems existed only with beet sugar and not with cane. I was then surprised in the early 1970's to learn that cane sugar had the same problems - not with saponins but with polysaccharides. Therefore, I am not so sure that you have foaming problems only with beet sugar.

We now have a method to determine the saponin content in juices. We have found that in raw juices amounts vary between 400 and 800 milligrams per kilogram of dry substance, and in thick juices between 5 to 9 milligrams per kilogram of dry substance. We are not yet sure if our method is also good for determining the amount in white sugar.

THE NEW REGENERATION SYSTEM (NRS) FOR DECALCIFICATION OF THIN JUICE

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Presented by R.W. Percival, Rohm and Haas Co., Latin-American Region

INTRODUCTION

The decalcification of thin juice is a very necessary step in the processing of beet sugar. The thin juice is concentrated from 10% - 15% solids to approximately 65% solids by evaporation, which represents one of the most expensive steps in the process. Therefore, to prevent calcium deposition on the evaporation tubes and maintain optimum heat transfer the thin juice must be softened prior to evaporation. The application of ion exchange materials has proven to be a very effective means of accomplishing this decalcification. A novel regeneration system is described which offers high regeneration efficiency, low cost, virtually no loss of sugar, and no regeneration waste streams.

DISCUSSION

A strong acid cation resin, such as Imac C-12, can be operated in the sodium form to decalcify thin juice beet sugar. Typically, an operating capacity of 0.6 to 0.7 Eq/L of wet resin is observed with this material. A minimum bed depth of 30 inches of resin is required for proper operation, where up to 90% of the hardness can be removed from the thin juice. There are several methods available for the regeneration step, which returns the resin to the sodium form for the next service cycle.

Brine Regeneration

Brine regeneration can be employed to regenerate the strong acid cation resin back to the sodium form. However, several problems exist with this method of regeneration, including the loss of sugar during the sweetening on and off step, the high regeneration dosage required to put the resin back in the sodium form and the creation of a regenerate waste stream. The column must first be sweetened off before contacting the resin with the 10% sodium chloride solution. Typical regeneration level is 200 g NaCl/L

resin 3.5 Eq/L, which is 500% more than the exchanged calcium. This is a result of the relative affinity of the resin for calcium over sodium. The spent regenerant cannot be used for any other purpose, so it must be disposed of, possibly posing an environmental issue.

Gryllus Process

The Gryllus Process can also be used to regenerate the resin. Once the thin juice has been decalcified and concentrated, the sugar syrup contains a high level of sodium ions. Therefore, the sugar solution can be used for the resin regeneration, instead of brine. This brings four main advantages:

- no chemical consumption
- as all rinsing and backwashing sequences are made with thin juice, there is no sugar loss
- as no water is used, there is no dilution
- no waste waters have to be treated.

Since calcium removed from the thin juice ends up in the final molasses, the Gryllus process can be applied in the sugar factories where molasses are not treated with the ion exclusion process, whereas the NRS process is highly recommended in the factories applying the ion exclusion chromatography.

New Regeneration System

The New Regeneration System (NRS) has been developed to address problems associated with the other methods of regenerating strong acid cation resin for thin juice decalcification. Generally, the technique involves the addition of sodium hydroxide to decalcified thin juice regeneration of the cationic resin. The use of NaOH alone is precluded by the fact that calcium hydroxide will precipitate immediately when the calcium ions are exchanged from the resin. However, when the sodium hydroxide is added to the thin juice and uses for regeneration, a soluble complex of calcium saccharate is formed. The spent regenerant is recycled to the first or second carbonation step. This results in a higher pH in the carbonation step due to the excess caustic soda present, which in turn enhances calcium precipitation, thus lowering the calcium load to the ion exchange resin. Other advantages to such a process include no sugar loss, no waste water, no juice dilution, low operating costs, and extended resin life. There is no sweetening on or off, so sugar is not lost during regeneration. Since the spent regenerant is sent back to the carbonation process, so no waste streams are generated. Unlike the Gryllus Process, there is no dilution of sugar syrups required. Lower operating costs are derived through the lower dosage of sodium hydroxide required for regeneration as compared to the use of brine. Resin life may be extended since there will

be no sweetening on and off. During this time, the resin experiences osmotic stress, which can result in bead attrition. The NRS can be conducted in both co-current and counter-current modes of operation.

The New Regeneration System Method

In practice, the NRS regeneration is carried out as follows:

1. Exhaustion of the resin is complete.
2. Backwashing the resin begins very slowly using thin juice at 90°C and the resin bed is air sparged to facilitate the fluidization of the bed. The flow rate of the thin juice is increased to remove any material which has accumulated on the resin bed. The juice used for backwashing is recycled to the filtration step.
3. Cooling of the resin bed is accomplished by passing approximately one-fourth of a bed volume of thin juice at 40°C through the resin in 15 minutes time. This step is necessary as insoluble saccharate could form at the higher temperature. The displaced juice is recycled to the filtration step.
4. Regeneration of the resin begins when one bed volume of decalcified thin juice, to which 40 grams per liter of sodium hydroxide has been added, is injected into the resin bed. The temperature of the regenerant is maintained between 40 and 50°C. The contact time is one hour. The spent regenerant is recycled to the first or second carbonation step.
5. Displacement of the regenerant is achieved by passing one-third of a bed volume of decalcified thin juice through the resin bed in 20 minutes. This juice is also recycled to the carbonation step.
6. Rinsing the resin is done with three bed volumes of thin juice which has not been decalcified. The juice is sent to the first or second carbonation step for recycling.
7. The service cycle now begins with no sweetening on.

Table 1.

A Comparison of Regenerant Consumption
Between NaCl and NaOH

<u>Regenerant</u>	<u>NaCl</u>	<u>NaOH</u>
Quantity	1250 Kg	125 Kg (190 Kg of which 65 Kg are recycled)
Dilution by Sweetening-Off	1600 Kg steam	None
Sugar Losses by Sweetening-Off	50 Kg	None
Cooling of Juice from 90 to 40°C	None	100 Kg steam

Table 2.

Advantages of the NRS Process

- Extremely simple operation.
- Short regeneration time.
- No fluctuation of the evaporator operating conditions due to sweetening-on and sweetening-off.
- No risk of chloride intrusion into the juice (resulting in corrosion)
- No regenerant waste water.
- Lower calcium feed concentrations to cation exchange resin.
- No risk of sugar losses through misoperation.

CONCLUSIONS

The New Regeneration System, as described, is capable of providing high regeneration efficiencies for strong acid cation resin used in the softening of beet sugar thin juice. Advantages to this regeneration technique include no loss of sugar, no need to sweeten on or off, low operating costs, and no regenerant waste disposal problems.

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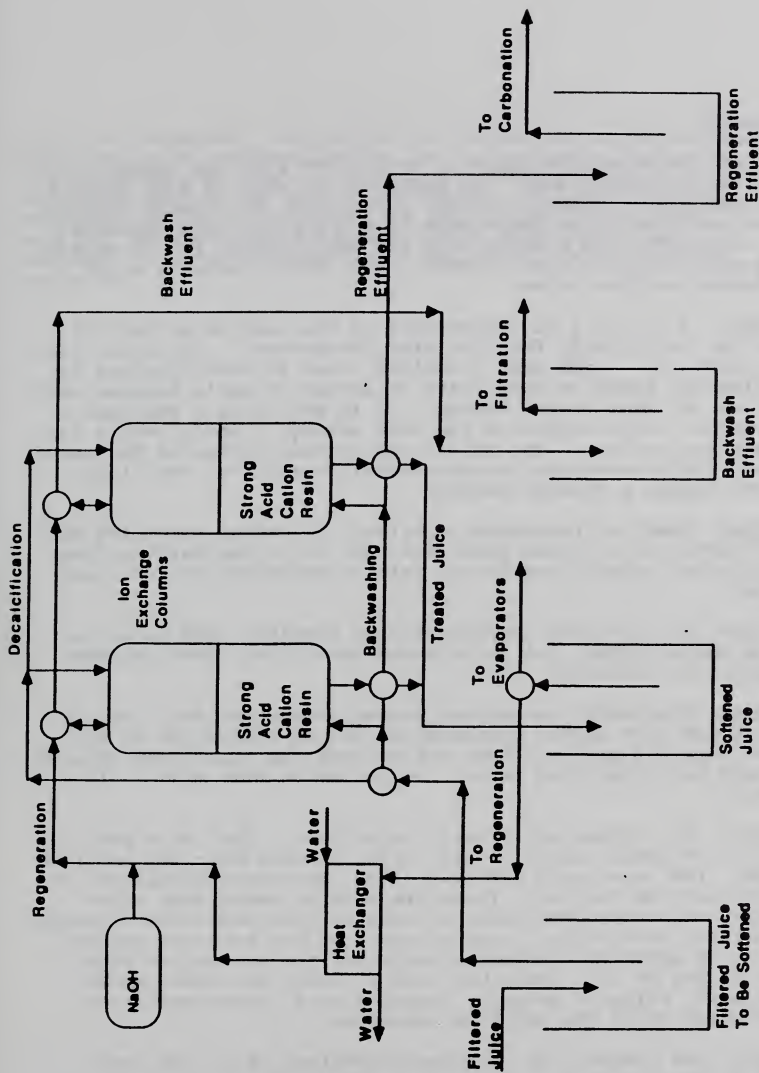


Figure 1. Schematic Diagram of NRS Installation

DISCUSSION

Question: We have some years of experience with the regeneration system using caustic soda. My question is, what is your opinion about cost effectiveness? In our experience, the system certainly does not save costs comparable to the normal brine regeneration. It solves the problem with the environment, but it causes more sugar losses to the molasses. The caustic soda is a bit more expensive than brine.

Percival: I will try to answer that to the best of my ability since I am not totally familiar with the process. To answer your first question: there are, I believe, over 40 installations in operation for three or four years in Europe; I don't believe that there are any here in the States. It is primarily a European development. With regard to the cost saving, I don't really see a tremendous savings. The benefit is an elimination of the waste problem. With increasing environmental regulations and fines, this may become a greater saving.

Question: When you regenerate up-flow with sodium hydroxide with caustic soda, in a cation resin bed that is in the calcium form, don't you have some formation of calcium hydroxide in the regeneration?

Percival: No. You are forming calcium succinate. The regeneration is not in water, but in softened thin juice, that is used throughout the process.

Question: What about co-current versus countercurrent? One of the problems with upflow regeneration can be levitation of the bed, so that you are in effect not putting the regenerant through the resin but around the resin. Do you solve that with a distributor?

Percival: No. There are several techniques. That is a good question: if people don't use the right process they get terrible results. Yes, you could get levitation, and there should not be any expansion of the bed. There are several techniques which keep the resin down: one would be counter-flow with water through an interface distributor; another would be air holddown; another would be to generate countercurrently and operate upflow with a screen on top of it. There has been a recent new development, "Amberpack", which is actually operated in a compartmentalized column which keeps the bed from expanding.

Comment: The interest of this new technology is to NRS, especially in view of what is happening in the beet sugar industry today, particularly in the United States, where the technology of ion exclusion for extracting sugar from molasses is developing extremely fast. NRS technology looks like the most elegant system to have soft molasses which will be suitable for that ion

exclusion treatment. If juices are not softened, the molasses will have to be softened before the ion exclusion treatment, and this would be very costly, of course. If the NRS system is used in the process of the beet sugar factory, then the system is improving factory operations, and also producing molasses suitable for ion exclusion treatment. It's very good technology, especially, in view of the increased extraction through ion exclusion.

CURRENT PRODUCTIVITY AND FUTURE OPPORTUNITIES FOR SUGARCANE AGRICULTURE FROM A PLANT BREEDER'S PERSPECTIVE

Don J. Heinz

Hawaiian Sugar Planters' Association

Presented by Benjamin Somera

INTRODUCTION

Significant improvements in yield have been achieved since sugarcane breeding began 100 years ago. Growers in developed countries have taken advantage of improved varieties through breeding, while those in less developed countries have lagged in adopting new, improved varieties selected for specific environments. Much progress--as much as 2 percent per year--in yield improvement can be achieved through conventional breeding programs.

Biotechnology, genetic engineering, offers new methods for the plant breeder to improve the crop. Adapting these methods is desirable to ensure continued rapid progress in developing varieties better adapted to specific environments with the potential for increased tonnage, sucrose content, disease, insect, herbicide, and stress tolerance. Maximum gains will come through the team effort of plant breeders and molecular biologists.

My presentation will be directed in three areas: (1) current productivity, (2) the basis for current productivity, and (3) opportunities for increased future productivity. I will indicate where biotechnology may play a role in sugarcane improvement through *in vitro* genetic manipulation.

Current Productivity

A comparison of today's yields with those of 25 years ago provides guidelines as to whether yields are increasing. Valid estimates of yield are confounded by local conditions which are hard to assess: droughts, rainfall, frost, hurricanes, and other factors which are part of an uncontrollable part of the environment. The contribution varieties make to yield are affected by growth regulators (ripeners, flower controllers), crop-protection chemicals (insecticides, herbicides, fungicides), and cultural practices such as fertilization, irrigation, soil pH control, and drainage, all of which, when properly applied, can modify the effects of the uncontrollable part of the environment.

Yields per hectare are given in the literature for tonnes of cane, tonnes of sugar and the ratio of tonnes cane/tonnes of

sugar for quality ratio. However, for our discussion, we will concentrate on the tonnes of sugar per hectare yields since this is a function of the other two and, in many instances, tonnes cane is not known, but is an estimated figure as in the Hawaiian industry.

Shown in Table 1 are the metric tonnes sugar per hectare for three areas in the United States, for Queensland, and for the Burdekin in Queensland, which is one of the prime sugarcane-growing areas in the world. The year figures in brackets are the years for the yield data which represent the highest yield for the period. The data for Hawaii are shown for the highest yields attained, 1958 through 1986, since there were severe droughts (1961 and 1962) in unirrigated areas and a prolonged labor strike in 1959, which severely affected yields.

The gains shown in Table 1 are modest and there are many reasons for the seeming lack of progress. Complicating the analysis, for example, is the fact that in Florida and Queensland, acreage has expanded by two to three times while, in Hawaii it has declined.

Table 1.--Sugarcane. Metric tonnes sugar per HA per year.

(Shown for a 25-year period where possible, except for Hawaii where the highest yield for the period 1958-1966 was chosen, since drought and strikes affected yields during this period.)

	1960		1984/85		% Increase
U.S. ¹					
Florida	7.34	(1960)	8.21	(1985)	12.0
Louisiana	4.88	(1960)	5.23	(1985)	7.0
Hawaii ^a	12.96	(1966) ^b	14.03	(1986)	8.0
Hilo Coast	9.49	(1958) ^b	13.00	(1986)	37.0
Irrigated	15.42	(1966) ^b	15.65	(1986)	1.5
Australia ²					
Queensland ^c	8.64	(1960)	8.83	(1983)	2.2
Burdekin ^c	12.37	(1960)	14.84	(1983)	20.0

^aAverage crop age in Hawaii is 24 months--figures presented are for one year.

^bSame hectareage and fields as area harvested in 1986.

^cAverage crop age in Australia is 13 months--data presented are for one year.

¹HSPA Hawaiian Sugar Manual.

²Australian Sugar Year Book

The yield curve (Fig. 1) for Hawaii from 1950 through 1986 highlights a problem when assessing only average yields. Although the state average yield held steady in the range of 10 to 11 tonnes sugar per hectare during the period 1966 through 1982, the leeward yields were declining because of poor cultural practices and smut disease. The yields on the Hilo-Hamakua Coast were increasing, which was attributed to new varieties, H59-3775, H70-0144, and H68-1158, since cultural practices were excellent during the period.

Shown in Fig. 2 are relative yields of sugarcane worldwide. Since 1980, there has been a decline in yield of about 15 tonnes sugar per hectare, with yields plateauing at about 5.1 tonnes of sugar per hectare, probably do to the expansion into poorer soils and environments. Expansion into new areas is usually based on the use of varieties that are not adapted to that environment. This suggests to me, based on experience, that breeders have the opportunity to increase yields with new varieties adapted to adverse conditions.

The contribution genetic improvement has made to increased cane sugar yield was estimated by Baver (1963) to be 75 percent of the yield increase attained by the Hawaiian industry in the 1950s.

Hogarth (1979) discussed the contribution to increased yields by new varieties in the Mulgrave Mill Area, Queensland, showing a 31 percent increase in sugar from 1964 to 1970-74. Pindar comprised 46 percent of the area in 1964, hardly any in 1970-74, while Q90 comprised 65 percent of the crop in 1970-74. He further argued that improved ratooning ability in new varieties allowed an increased number of ratoon crops from a 1:1 ratio in 1948 to a 1:2.9 ratio in 1975. He calculated that, in Queensland, yields increased 1.9 percent per annum from 1948 through 1975, of which 50 percent might be attributed to genetic improvement.

In Hawaii, yields have improved every decade except the 1970s when disease and agronomic problems plagued the industry (Fig. 2). Through development of new, improved, disease-resistant varieties and the introduction of drip irrigation, yields of sugar increased again in the 1980s. A large gain in sugar yield was obtained on the Hilo-Hamakua Coast of Hawaii from 8.96 mt/ha/yr in 1955 to over 12.32 mt/ha/yr from 1983 on--a 3.36-tonne improvement. It is likely that at least two-thirds of the gain was due to improved varieties (Heinz, 1987).

Breaux (1984) reported an improvement in sucrose content in Louisiana varieties through use of a recurrent breeding and selection program of 9.1 to 13.5 percent in five cycles of breeding.

Disease Resistance

Probably of equal importance to sugarcane growers are the maintenance of yields and the selection of disease-resistant varieties through breeding. Three excellent examples from the past 15 years can be cited.

Rust disease. Economic losses of up to 25 percent of production occurred in the most recent Caribbean-American outbreak on several major commercial varieties, notably B4362 and Cl 41223, that were susceptible to rust (Walker, 1987). There was a 25-50 percent reduction in yield in variety H54-775 in 1983 and 1984 in Hawaii, although only on about 800 hectares. More important, a potentially high yielding variety (up to 10 percent increase) on the Hilo Coast had to be discarded because of high susceptibility to the disease.

Smut disease. Varieties highly susceptible to smut can be reduced in yield by 20 percent (Walker, 1987). In Hawaii, Pioneer Mill Company suffered at least a 20 percent reduction in yield as measured by plantation yields before and after smut on the susceptible variety H50-7209 (Table 2). In 1980 and 1981, 100 percent of the harvested area was planted to H50-7209. By 1983, 100 percent of the harvested area was planted with smut-resistant varieties, H62-4671 and H69-8235, indicating a reduction of at least 20 percent.

Table 2. Yields at Pioneer Mill Company, Hawaii, on variety H50-7209 prior to heavy smut infection affecting yields in 1980 and 1981 and harvesting smut-tolerant varieties in 1982 and 1983.

Year	<u>Mt/sugar/ha</u> (2-year crop)
1979	25.9
1980	24.2
1981	22.5
1982	28.1
1983	29.6

Fiji disease. Fiji disease has caused as much as a 20 percent decrease in yields in the Bundaberg sugarcane growing area of Queensland (Anonymous, 1982). Losses occurred in highly susceptible NCo 310. The disease outbreak has been controlled through the planting of disease-resistant varieties.

Through the use of new higher-yielding, disease-resistant varieties, sugarcane breeders have provided farmers the opportunity not only to maintain yields but also to increase yields. Because of expansion into poorer growing areas, overall sugarcane yields have not increased in proportion to those shown for other crops. However, while the sugarcane acreage generally has been expanding, corn acreage in the United States has decreased with the shrinkage taking place in the poorer areas.

The expansion into new areas with varieties not adapted to those environments offers opportunities for the breeder to make rapid progress in breeding and selecting varieties adapted to those conditions.

Development of Modern Sugarcane Cultivars

The developments that led to modern sugarcane cultivars were reviewed by Jeswiet (1930). Workers in Java and Barbados initiated intraspecific crossing in an attempt to improve sugarcane. Walker, in 1893, open-pollinated Bandjermasin Hitam (*S. officinarum*) to produce POJ 100. During the same year, he crossed Cheribon (*S. officinarum*) with Kassoer, a wild cane which Jeswiet later recognized as a hybrid between *S. officinarum* and *S. spontaneum*.

From 1916 through 1920, Jeswiet backcrossed Kassoer seedlings to *S. officinarum* without success. However, out of crosses made in 1921, he obtained 2,266 seedlings from which he selected POJ 2725 and POJ 2878. POJ 2878 was planted in over 400,000 hectares in Java within eight years, having Sereh disease resistance and an average yield gain of 35 percent over its *S. officinarum* predecessors. POJ 2725 and POJ 2878 are in the pedigrees of almost all the world's cultivars of sugarcane.

The genetic basis for yield gains in sugarcane is the cross between *S. officinarum*, the sugar producing species which was found only in native gardens in Indonesia, and *S. spontaneum*, found in many tropical and subtropical areas around the world. This cross produces progeny with two genomes from the *S. officinarum* and one genome from the *S. spontaneum* to produce $2n+n$ progeny. As far as I can determine, there are no varieties in production today that have not originated from this base.

The complexity of modern day varieties is indicated by the pedigree of H65-7052 (Fig. 3), the top variety in Hawaii. At least three species, *S. officinarum*, *S. spontaneum*, and *S. barberi* (the latter two of the Sarethia group) are involved in the parentage of this variety. Additionally, there may be chromosomes from *S. robustum* (Port Moresby type) and *S. sinense* (Pansathi group) since other prominent Hawaiian varieties, including H37-1993, H50-2036, and H57-5174, were used freely in polycrosses.

Most of the genetic gain for improved yields and disease resistance has come through intercrossing clones developed from the POJ and Co varieties, based on a few *S. officinarum* and *S. spontaneum* clones. Berding and Roach (1987) summarized the information on the collection, maintenance, and use of sugarcane germplasm. They were not able to list any new sources of germplasm used for the development of varieties other than the original used in hybridization in Java and India in the early part of the twentieth century. They concluded that "...it seems inconceivable that further hybridization has nothing to contribute. If further gains are to be made from nobilization, then procedures will need to become more sophisticated. It is unlikely that small populations of seedlings produced with the broad objective of adding genetic variability from unselected germplasm will provide varieties superior to those presently grown."

Future Improvements

Improvements in sugarcane yields can come through breeding and selection from the current germplasm pool. In fact, I expect improvement in the leeward Hawaii environment to come from within our present germplasm pool since the microenvironment has been stabilized through drip irrigation and excellent cultural practices. Continued improvements will be achieved in other areas of Hawaii, but gains will not be achieved as easily as in the past.

Long-term improvement in yield will come through the use of all available technology, including conventional and molecular techniques. However, in areas where a viable breeding program has not been in place, rapid improvement can be made through a commercial breeding program. Discussed below are a few areas I perceive to be of significance to sugarcane breeders.

New Techniques and Application

The transfer of genes from one species to another has been achieved many times in the past 75 years. Sugarcane is a good example. Goodman, et al., (1987) listed examples of agriculturally important genes and traits transferred to crop plants by interspecific or intergenic hybridization. Two families dominate the list, *Graminae* (wheats, oat, rice and maize) and the nightshade family, *Solanaceae* (tomato, potato and tobacco). In their limited list, examples are shown of over 40 traits that have been transferred.

Examples of transgenic (DNA from one species to another *in vitro*) plants are tomato, potato, petunia, tobacco, carrot, celery, cotton, flax, alfalfa, lettuce, sunflower, rape-seed oil, cabbage, asparagus, cucumbers (Fraley, 1989). Personnel at Monsanto Chemical Co. are working toward the release of soybeans resistant to the herbicide glyphosate and tomato varieties resistant to glyphosate and tobacco horn worm (Fraley, 1989). We can expect

other transgenic crop varieties to be developed and released using high protein alfalfa and cereals. The greatest progress in the development of transgenic plants may be with varieties having cross viral, fungal or bacterial resistance, insect resistance, and herbicide resistance. Although the new biotechnology will not displace the older plant breeding methodologies, successful plant breeders will have to be part of a team that will integrate all technologies to combine desirable payoff. Progress on complex characters in most plants will be slow because of our basic lack of understanding, metabolic, physiological, and biochemical pathways. We will need a much better understanding of these pathways to make progress in producing transgenic varieties with yield potential touted in many publications. It should be emphasized that no new varieties have yet been released that were developed through genetic engineering or are there significant varieties that have been developed through tissue culture techniques.

Research at HSPA has for many years included the use of cell, tissue and callus cultures, and the expertise developed as a result on this work helps set the stage for many of the advances we are hoping to achieve over the next few years.

Following are examples directly concerned with the relevant techniques presently under investigation at HSPA.

Somaclonal Variation

Our first attempts at *in vitro* improvement of sugarcane began in 1962 with the production of callus tissue. In 1967, we were able to produce totipotency and derived numerous plantlets from a number of clones. We noted variation between plantlets and screened large population changes in reaction to the following diseases: eyespot, smut, rust, Fiji disease (in Fiji), and for a higher sucrose content. Variation was genetic and non-genetic in origin. Most of the genetic variation was due to loss or addition of whole chromosomes. We did not recover any useful varieties from this work, but it set a firm foundation for the work we want to do in the area of biotechnology, especially micropropagation, haploid production and transgenic plant production.

Micropropagation

Our Indian (SRI) and the (PLANALSUCAR) Brazilian colleagues (PLANALSUCAR) demonstrated that micropropagation, using meristem or shoot tip culture would be a very effective method of rapidly propagating a new clone, with no expectation of somaclonal variation, since there is no callus phase. We became interested in micropropagation, not only as a tool for rapidly propagating a new clone, but also as an alternate method of commercially propagating sugarcane. One plantation was commercially propagating sugarcane to transplant two-month-old plants originating from

bud slices. They were spacing their plants 1.5 m apart in 1.5 m rows. Using transplants, seed farm requirements would be only one-fiftieth, or 2 percent of that formerly needed, so that the remaining area could be used for sugar production. Yields from transplanted fields are equal to yields in fields planted in the standard way.

Protoplasts

The regeneration of sugarcane plants from protoplasts will provide opportunity for gene splicing and transformation of the sugarcane genome. Encouragement comes from reports by Tabaeizadeh et al., (1986) and Yan et al., (1985) that plants have been regenerated from sugarcane protoplasts. Vasil and Vasil (1980), Lu et al., (1981), Vasil et al., (1983), and Vasil (1983) reported the regeneration of plants from several genera, including sugarcane. The first report of regeneration of plants from rice protoplasts dates back to 1985 (Fujimura et al., 1985; Coulibaly and Demarly, 1986; Yamada et al., 1986; and Abdullah et al., 1986), and these, as well as success with maize, suggest the barriers once thought to exist in plant regeneration from monocotyledonous protoplasts have fallen. Within a short period this technique should be available to sugarcane breeders. Protoplasts are one of the major routes toward somatic hybridization, gene transfer, and other genetic manipulations.

The regeneration of plants from protoplasts has been a barrier in achieving the introduction of foreign DNA via this system and expressing r-DNA-altered genetic characteristics in sugarcane. Until recently, only protoplasts could be manipulated for DNA uptake. In sugarcane, plant regeneration from protoplasts, even where it has been achieved, has been so minimal that it is useless for a successful selection of plants expressing new traits via stably integrated r-DNA. Recently, the method of bypassing the protoplast step and introducing DNA-coated gold or tungstate particles into whole cells with a high-velocity gun has proven to be more effective for obtaining stable DNA integration than any of the protoplast-based methods. So far, we have used this method in preliminary experiments with a number of different types of sugarcane cell cultures. A DNA construct that includes a kanamycin resistant gene to select only cells resistant to this antibiotic, and a β -glucuronidase gene which can light up transformed cells, has been employed. Regeneration of resistant plants is presently in progress, and plantlets will be assayed for β -glucuronidase enzyme expression as soon as enough leaf tissue can be excised without destroying the seedlings.

Haploids

Fitch and Moore (1983) were successful in producing haploids through anther culture from SES 208, a *Saccharum spontaneum* with $2n=64$ chromosomes. Plants with $2n=64$ and $2n=32$ chromosomes were

recovered from callus tissue derived from SES 208 anthers. Considerable variation was found among anther derived plants (C. Nagai, HSPA, personal communication). Isoenzyme studies of both populations showed the same isozyme (esterase and peroxidase) pattern variation which differed from SES 208. The similar variation in isoenzyme patterns among the $2n=32$ and $2n=64$ plants, suggests the $2n=64$ plants are doubled haploids. Isoenzyme variation was not observed in SES 208 somaclones. At meiosis, bivalent pairing was observed in both the haploids and suspected doubled haploids with occasional univalents, suggesting auto-syndetic pairing. Plants from both populations, even though they may be heterozygous, should be useful in genetic studies. Thirty haploid plants have been identified from SES 208, all flowering; two from SES 205 A ($2n=64$), both non-flowering; and one from US 56-15-8 ($2n=80$), non-flowering (personal communication, C. Nagai, HSPA and M.M. Fitch, USDA-ARS). Efforts are continuing to produce haploids from a wider range of clones, especially US 56-15-8 and close relatives, which are *S. spontaneums* from Thailand, showing promise as breeding material in Louisiana and Hawaii (Heinz, 1980; Dunckelman, 1978).

The production of haploids offers opportunities to better understand the genetics of sugarcane, especially when used with restriction fragment length polymorphism (RFLP) analysis. Although haploids have been produced from only three clones of *S. spontaneum*, the opportunity exists for deriving haploids from a wider range of clones in different species. Even with the few existing clones, it will be possible to determine the effect of a reduced chromosome number on the transmission of *S. officinarum* chromosomes when crossed with haploids or doubled haploids.

Restriction Fragment Length Polymorphisms (RFLP)

Restriction fragment length polymorphism allows recognition of segments within the DNA structure. Once a specific segment is identified, either directly or with reference to a similar sequence in another plant (maize or sorghum are particularly relevant for sugarcane) where the segment has already been correlated with a trait, it can be used as a selection tool for new varieties. It should then be possible to make quite accurate, rapid predictions for the behavior of progeny from a given cross. We have four objectives in a cooperative program with Cornell University and Centro de Tecnologia of Copersucar in Brazil: (1) To survey *Saccharum* species, sugarcane varieties and haploids lines for RFLPs; (2) Develop an RFLP map with sugarcane; (3) Develop protocols and adopt software for interfacing RFLP map information with our breeding program; and (4) Obtain training for Experiment Station personnel who will be involved in RFLP technology transfer to Hawaii.

This project represents the first effort anywhere in the world to apply RFLP technology for the improvement of sugarcane. We

expect this technology to play a significant role in our breeding program in three to five years. It probably will be the first use of the new technologies in the improvement of sugarcane and the one that will have the most significant impact.

Cytology

Knowledge of the cytogenetics of sugarcane is limited mostly to a reasonable assessment of the chromosome number for various species of *Saccharum* and some of the related genera. This has been valuable in characterizing the genus, but much more information is needed cytologically, morphologically, and physiologically before the various clones represented in the world collections of sugarcane can be adequately characterized. This information is needed to assist the breeder in making intelligent choices in intercrossing clones within the genus.

Most chromosomes in commercial hybrids are derived from *S. officinarum*, although the exact proportion is unknown (Roach, 1984). The phenomenon of the *S. officinarum* somatic complement being passed through to its progeny when crosses with *S. spontaneum* is significant since almost all commercial hybrids are based on $2n+n$ chromosome transmission. Greatest progress in "nobilization," the stepwise improvement of sugarcane from wild *S. spontaneum*, is dependent on the full somatic complement of *S. officinarum* in the progeny (Heinz, 1980; Roach, 1986). Important as this phenomenon is to rapid genetic gain, very little is known of the mechanism driving it. Sreenivasan et al., (1987) summarized the theories purported to explain the mechanism whereby full somatic complementation occurs, but only Bremer (Sreenivasan et al., 1987) actually attempted to obtain physical evidence to explain the process. A better understanding of this mechanism might assist breeders in making greater progress utilizing other species and genera other than *S. spontaneum* in the improvement of sugarcane. If the mechanism were hormonal, it might be identified and used in forcing $2n$ transmission by *S. officinarum* when crossed with other species.

Herbicide Resistance

Culturing on media containing an herbicide to select resistant cell variants has been a method successfully used for several years with other plants. Periodically, by various groups, there have been abortive attempts to apply similar methods with sugarcane cultures. Until recently these efforts were not consistent, and problems were encountered in regenerating large numbers of the surviving cells. We have now overcome these problems and the method is being used to select against broad spectrum sulfonylureas and a glyphosate-like herbicide marketed by Hoechst & Co. Multiple exposure to threshold, lethal concentrations of the herbicide are being used before apparent resistant colonies are regenerated to plants. The plantlets do not all survive, but a

sufficient number are surviving to conduct tests which will determine whether increased resistance has been achieved. Future selection will include treatment with a chemical mutagen prior to stress selection.

The objectives under (2) will include the transformation of sugarcane cells to impart specific herbicide resistance, and suitable DNA constructs available from industry will be used for this purpose.

Through a double-pronged approach, useful herbicide-resistant sugarcane varieties should be commercially available within a few years.

SUMMARY

Increased sugar yields through breeding have come through crossing clones derived from a limited sample of all germplasm available in the genus *Saccharum*. Disease-resistant, stress (herbicides, drought)-tolerant clones have been derived from the same limited germplasm pool. Further progress can be made by crossing within this same germplasm pool.

Interspecific hybridization offers opportunities for sugarcane improvement if the crossing scheme is properly planned and executed. This holds promise, especially in developing higher-yielding clones in new environments, such as high elevations or in areas exposed to stress, i.e., drought, cold and insect pests.

More attention needs to be placed on selection for defined environments, especially where agronomic practices have been optimized. Selection in less favorable environments will pay dividends.

New techniques in plant biotechnology are not intended to replace conventional breeding methods, but rather to facilitate and supplement them.

The successful breeder in future years will utilize all available tools to provide yield increases to be obtained under optimized growing conditions.

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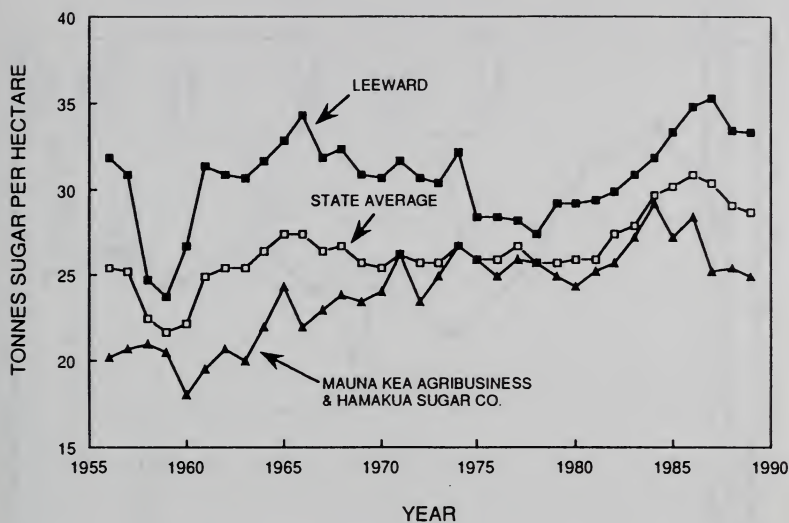


Fig. 1. Tonnes sugar her hectare 1955-1989 for the Hawaiian sugar industry. The three curves represent the leeward (irrigated) plantations, the state average for all plantations, and the Hilo-Hamakua Coast plantation yields.

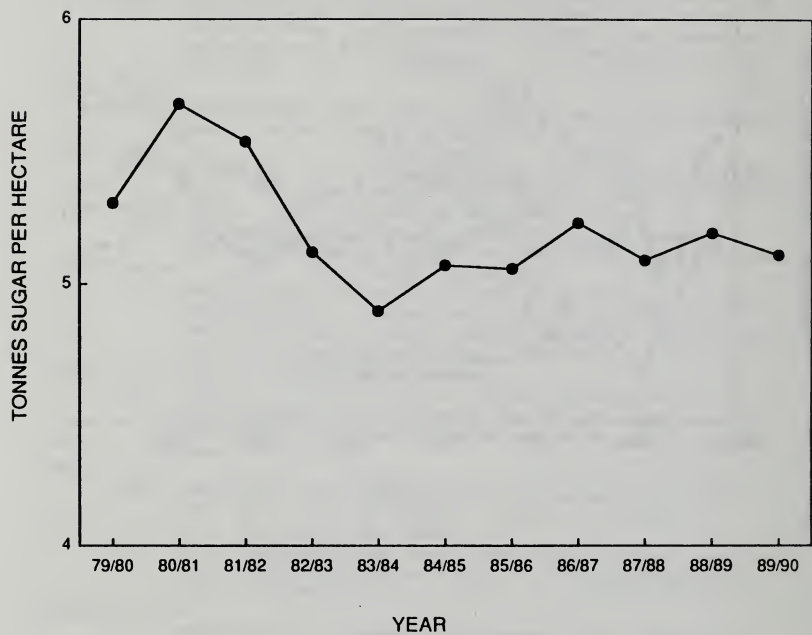


Fig. 2. World average yields of cane sugar.

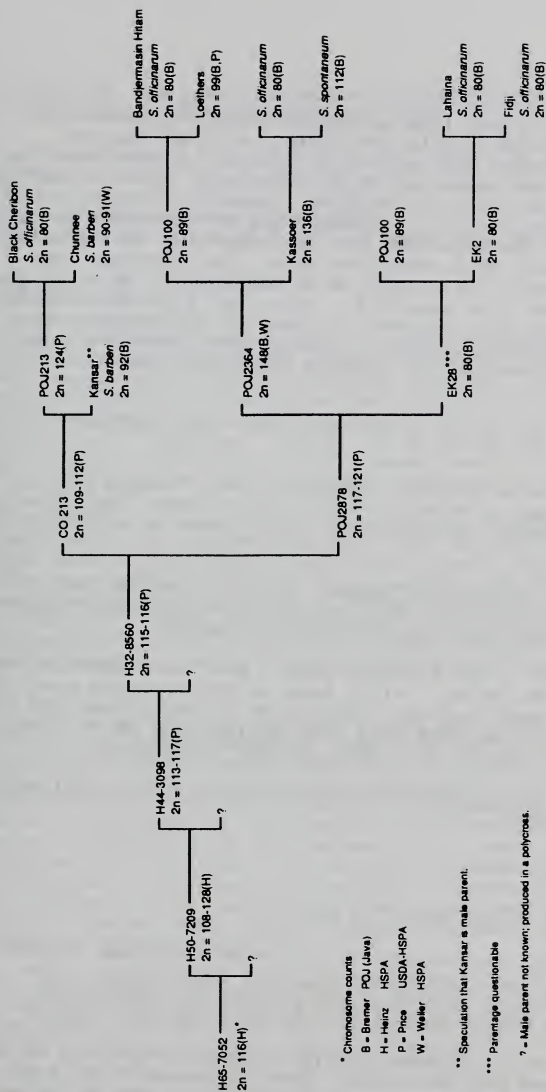


Fig. 3. The pedigree of H65-7052 showing the derivation of this cultivar and the complexity of its parentage. In sugarcane pedigrees the female is always shown on the top line. The chromosome number is given for each clone, with the letter in parenthesis indicating the individual making the count.

DISCUSSION

Question:⁽¹⁾ I would first like to congratulate Ben Somera on handling of a paper out of his own field. As I am also an engineer, I won't argue with any of it either. I know that the plant breeders in the Bureau of Sugar Experiment Stations have a great respect for the work of the HSPA. However, I would sound a word of caution when you try to compare yields from area to area because you have to be sure that you are comparing apples with apples and not apples with something else. For instance, the Australian crop (and I don't want to be parochial), is a one-year crop, not a two year like Hawaii's. Also, our figures of tones of sugar per hectare are reported as tonne of recoverable sugar and not as simply tonnes of pol. I think that these are aspects you must consider when you are looking at yields from area to area.

Somera: I'll ask Dr. Heinz to be sure that they are comparing apples with apples.

Chairman: There is no question about it, the ultimate goal is to increase per acreage at present in the area planted, but also to provide more adaptable varieties for other areas we have to plant in the future.

Question:⁽²⁾ A most interesting paper. Do you think the new varieties you are developing have any surprises for the refiner in terms of the non-sugars which might be created?

Somera: I'm afraid I can't answer that. That is a question that I will direct to Dr. Heinz. All I know is the breeders are trying to insure sugar content, resistance to herbicides and to diseases. Other places have used cane millability as a criterion, but in Hawaii, as far as I know, we don't use that.

Comment: We'll look forward to receiving them at the refinery, and will let you know.

Answers, provided by Dr. Heinz by mail.

- (1) Yield data for two periods in each area are given to plot progress or lack of progress in yield improvement. The data are given on an annual basis in metric tonnes per hectare, but not corrected for raw or commercial sugar.
- (2) New varieties grown commercially in Hawaii generally do not cause problems for the mills. Some adjustments are made at the mill to maximize recovery of sugar from new varieties.

OPTIONS FOR AND IMPLICATIONS OF INCREASING THE SUPPLY OF BAGASSE BY INCLUDING TOPS AND TRASH WITH CANE

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INTRODUCTION

Much of the world's sugar cane is grown in countries where rapidly increasing populations are creating a growing demand for basic commodities such as building material, feedstuffs and paper. Bagasse is a suitable raw material for such commodities and it is expected that the economics of using bagasse for these purposes will improve. Indeed, in South Africa much cane land has recently been planted with timber thus proving that there is a lucrative market for cellulose-based products.

The potential to increase future revenue from bagasse comes at a time when it is increasingly difficult to improve revenue from sugar because high efficiencies are already being achieved. This makes it logical to divert some research and development effort from sugar processing towards better bagasse production and utilisation. Again, in South Africa, this logic is substantiated by the fact that at 3 factories bagasse supplies have ceased to meet by-product demand despite extensive use of coal as boiler fuel.

Perception of these trends means that personnel who are involved with the processing of sugar cane have had to shake off the traditional understanding that their work starts at the factory gate. The handling of the crop between the field and factory is open to various options which have considerable impact on the overall processing of cane, particularly on the production of fibre. Ironically, at a time when it is appropriate to increase supplies of fibre there is a strong awareness among cane millers of the need to improve cane quality by reducing extraneous fibre. The issues of cane quality and fiber supplies are intermeshed and require further study before optimum systems can be selected for different circumstances.

This paper deals with preliminary studies done by a combined team of agricultural and factory personnel and which are aimed at generating data on different harvesting techniques and their impact on sugar recovery and fibre supply.

METHODS

Harvesting and transport

Cane was harvested manually using the following procedures for the different trials:

- Trial 1. unburnt, topped (but not trashed) (UT)
- Trial 2. burnt and topped (BT)
- Trial 3. unburnt, not topped nor trashed (UU)
- Trial 4. burnt, not topped (BU).

The cane was variety NCo376, aged 19 to 20 months and with an average yield of 120 t/ha. It was all harvested from adjacent fields but there were two-week intervals between each trial. To minimise the effect of this time delay the trials were done in mid-winter (when there is minimum growth but no frost). The harvested cane was manually piled into small bundles which were loaded into 5-ton field trailers with a Bell grab loader. The contents of 5 field trailers were later loaded into a single Hilo truck for transport to a mill. Each trial involved 8 such Hilo trucks.

Before loading, the small bundles in the field were samples by taking 5 stalks per bundle from approximately 10% of the bundles. These samples were then composited to give samples containing 20 stalks. The composite samples were analysed for brix, pol, fibre and dry matter. In addition stalks from each sample were carefully stripped of all trash and if not topped, or if topped too high, tops were broken off at the natural breaking point. Trash and tops were weighed separately and their dry matter contents determined. In trial 3 they were also analysed for brix, pol and fibre.

Processing

To simulate commercial practice in South Africa, processing of the cane took place 3-4 days after harvesting. A milling tandem, rather than a diffuser, was used for extraction because the latter requires more than 200 t of cane to achieve equilibrium.

During processing, measurements were made of cane throughput, motor loads on cane knives, steam flow to turbines and first mill torque. Samples were taken of stalks from the cane carrier, shredded cane from the "payment" sampler, mixed juice from the "payment" sampler and final bagasse from a full-width hatch.

The sampled cane stalks were hand cleaned, topped and trashed and then sub-sampled and analysed to provide a standard "background" analysis for each trial.

Computer model

A computer model was developed to predict outputs of sucrose, molasses and bagasse based on inputs of various proportions of tops, trash and stalk of known composition. The predictions are based on extraction and recovery formulae which have been developed for South African conditions and which take into account the cane composition in terms of sucrose, non-sucrose, fibre, reducing sugars and ash.

The extractions are estimated from a corrected reduced extraction (CRE) formula (SASTA, 1985), using an assumed CRE of 97.5% and calculating actual extractions for the different cane qualities. Recovery calculations are based on a molasses target purity formula (Ravno and Lionnet, 1982) related to mixed juice quality. The actual purity of molasses is assumed to be 6 units above the target purity as calculated from mixed juice. An undetermined loss of 2% is assumed.

The calculation of bagasse surpluses simply assumes that imbibition % fiber is constant for all four trials, the steam requirement is 50% on mixed juice and each ton of steam is generated from 0.5 t of wet bagasse.

The model also takes into account sucrose losses due to delays between harvesting and crushing (de Robillard *et al.*, 1990) and, based on measurements made during the harvesting trials, it calculates the costs of the various harvesting systems together with the associated loading and transport costs.

RESULTS AND DISCUSSION

The pol % clean stalk increased from 13.4 to 14.5 over the six week period of the trials thus making direct comparisons between trials slightly erroneous. Furthermore the cane involved in Trial 2 was drought stressed, making its fresh stalk mass relatively low and its pol % stalk relatively high.

Harvesting, loading and transporting.

The productivity of cutters and loading and transportation equipment is summarised in Table 1.

On a total mass basis cutter productivity was clearly highest with the unburnt untopped cane, being 42% higher than with the burnt topped (BT) cane. On a clean cane basis however the productivity was greatest with the burnt untopped cane (17.5% higher than for the BT cane). As expected the lowest productivity was for unburnt cane requiring topping (9.3% lower than for

BT). Topping of either burnt or unburnt cane slowed the harvesting rate by about 20% when measured on a clean cane basis (about 50% on a total mass basis). Burning increased cutter productivity by 4% when topping was not required and by 7% when topping was required.

TABLE 1.--Productivity of men and equipment working with different harvesting techniques

	Unburnt topped	Burnt topped	Unburnt untopped	Burnt untopped
<u>Total mass</u>				
Tons/man hour (cut and bundle)	1.02	1.06	1.51	1.33
Tons/trailer	4.16	5.17	3.60	4.99
Tons/hilo	20.79	26.04	18.16	24.95
Loading rate (t/h)	35.77	43.17	33.60	45.70
<u>On clean cane basis</u>				
Tons/man hour (cut and bundle)	0.93	1.03	1.17	1.21
Tons/trailer	3.76	5.00	2.80	4.55
Tons/hilo	18.77	25.21	14.13	22.75
Loading rate (t/h)	32.30	41.79	26.14	41.68

Loading and transport rates were affected substantially by the harvesting methods. Unburnt (untrashed) cane was loaded relatively slowly and gave very low payloads. For example, on a clean cane basis the unburnt untopped cane gave payloads 44% less than those with BT cane, and loading rates 49% less.

On a cost basis the influence of harvesting methods on cutting is small compared with the influence on loading and transport. The last two predominate in determining the total cost of harvesting and delivery, and for a relatively short delivery distance of 11 km the total harvesting and delivery costs, expressed as a percentage of those for BT cane, were:

burnt untopped	102%
unburnt (untrashed) topped	116%
unburnt (untrashed) untopped	133%.

Composition of delivered cane

The composition of cane derived from the different harvesting techniques is shown in Table 2.

Without burning, about 20% of the dry matter delivered to the mill was trash, and the fibre content of the delivered cane exceeded 21% whereas it was only about 14% after burning. This clearly illustrates the large potential for increasing fibre supplies by modifying harvesting techniques. The cane analyses show however that cane quality is affected substantially with purities ranging between 80 for the unburnt untopped cane and 88 for the BT cane. When all extraneous matter was removed from stalks sampled at the mill the stalks from the different treatments had a narrow range of purities (89.4 to 90.9) and fibre contents close to 12.5%.

TABLE 2.--Composition of cane harvested by different methods

	Unburnt topped	Burnt topped	Unburnt untopped	Burnt untopped
<u>% wet mass</u>				
Cane	90.3	96.8	77.8	91.2
Tops	2.8	2.7	14.3	8.3
Trash	6.9	0.5	7.9	0.5
<u>% dry mass</u>				
Cane	80.5	96.1	66.2	88.8
Tops	1.8	2.4	13.0	9.8
Trash	17.6	1.4	20.8	1.4
<u>Whole cane</u>				
Pol	11.53	13.57	10.63	13.39
Brix	13.61	15.35	13.23	15.50
Purity	84.63	88.40	80.36	86.37
Moisture	65.16	70.25	65.48	69.82
Fibre	21.22	14.40	21.58	14.68
Ash	2.48	1.13	2.76	2.11

Colour and turbidity

The trash contributed substantial colour and turbidity to the juice produced at the factory (Table 3). From the results it might seem that trash contributes much more colour than tops but the contribution of colour per unit of additional fibre is similar for tops and trash as indicated by the near linear relationship (above 15 000 colour) in Figure 1. Furthermore, colour is expressed on a unit brix basis, and because tops

contain much more brix per unit dry mass than does trash, the colour from tops is partially masked by its associated brix because the brix increased the denominator used in the calculation of colour. This point is emphasised because Reid and Lionnet (1989) and Ivin and Doyle (1989) stressed the fact that trash produced much more colour than tops; a point which can be misleading when considering the compromise between colour production and fibre production.

The unburnt untopped cane gave juice which would not clarify effectively so that the clarified juice had high turbidity. The nitrogen content of the juice was also increased by tops and trash (Table 3).

After the initial trials an opportunity arose to repeat part of the investigation using a diffuser instead of a mill. The results with respect to colour were remarkably similar - the unburnt untopped cane giving mixed juice colour of 29 510 with the diffuser and 32 980 with the mill.

TABLE 3.--Characteristics of juices associated with different harvesting methods.

Trial No.	Mixed juice			Clear juice	
	Brix	Colour	Nitrogen	Turbidity	Colour
1 (UT)	12.50	23 660	0.257	3 800	22 090
2 (BT)	12.94	15 860	0.198	1 600	14 970
3 (UU)	10.09	32 980	0.305	9 030	30 730
4 (BU)	13.44	16 240	0.223	2 250	15 000

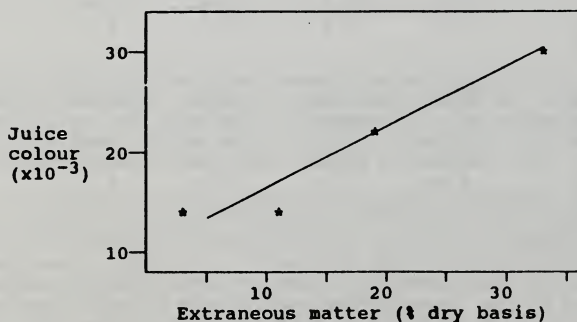


Figure 1.--Juice colour associated with different harvesting techniques and related to extraneous matter in cane

Factory performance

Capacity. The mill speed was maintained constant for all four trails. Capacity in terms of pol was seriously affected by the presence of extraneous matter whereas capacity for fibre was little affected (Table 4).

TABLE 4.--Throughputs associated with different harvesting techniques.

Trial No.	t cane/h	t fibre/h	t pol/h
1(UT)	140	30	16
2(BT)	181	26	24
3(UU)	127	27	13
3(BU)	190	28	25

Sugar, molasses and surplus bagasse production. Sugar recoveries have been calculated for the different mixed juice qualities (Reid and Lionnet, 1989). The results are not strictly comparable because of the change in cane quality between the first and last trials. An alternative estimate of the various end-products has been made by using the analyses of the unburnt untopped cane as inputs to the computer model and then simulating the various harvesting techniques to generate estimates of cane quality delivered to the mill. From these estimates the model predicts quantities of sucrose, molasses and surplus bagasse.

The calculated quantities of end-products from 100 t of standing cane are shown in Table 5.

TABLE 5.--Computed quantities derived from 100 t of standing cane when harvested using different methods.

	Unburnt topped	Harvesting method		Burnt untopped
		Burnt topped	Unburnt untopped	
Harvested cane mass	85	78	99	92
Sugar	8.71	8.82	8.89	9.02
Molasses	2.79	2.74	3.07	2.99
Surplus bagasse	8.10	-0.36	10.83	2.36
Relative profit	90	100	78	90

Relative to BT cane the unburnt untrashed cane would require at least an additional 27% transporting and crushing capacity. Much of the cane delivered in South Africa has an extraneous content similar to that of unburnt topped cane and it can be seen that if this was burnt and topped then the required crushing capacity would decrease by 9%, or the seasons length could be reduced.

Profits have been calculated for the overall growing and milling operation. Obviously the profits would vary widely according to particular circumstances, such as transport distances, so the values shown in Table 5 give only a vague indication of the impact of harvesting technique. Furthermore the calculations do not take into account the non-quantifiable costs associated with increased colour, and they are based on unit mass of standing cane, not delivered cane. They assume that there is a market for fibre at its coal equivalent value.

The calculations show that the highest profits are associated with the cleanest cane. It is interesting however to speculate on situations where bagasse has a higher value than its present coal replacement value, all other factors remaining constant. Relative profits under these circumstances are shown in Figure 2.

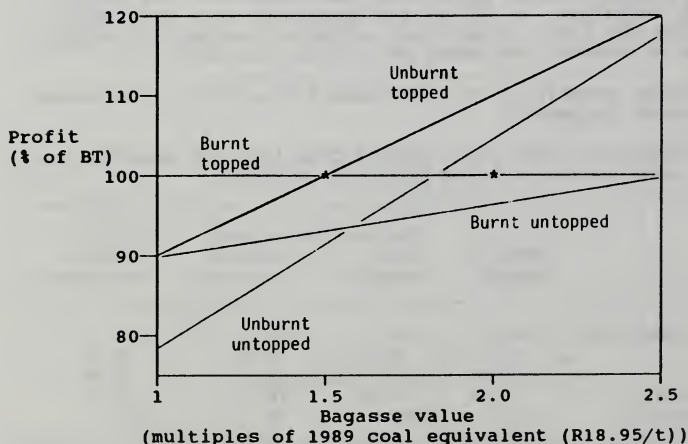


Figure 2.--Relative profits from different harvesting techniques for different fibre values

Where bagasse is valued against a 1989 coal price of R72/t the most profitable harvesting system involves burning and topping but if the bagasse value is more than 50% higher then it is likely than the unburnt topped system would be more profitable (Figure 2).

Whereas these profit analyses may be imprecise they do illustrate the potential for increasing revenue by selecting the appropriate harvesting system for particular circumstances.

Dry cleaning

The conflicting requirements of clean cane for the factory on one hand and high fibre cane for fibre sales on the other hand can possibly be reconciled by the introduction of dry cleaning at the factory. Until recently the incentive for dry cleaning has been solely to improve cane quality, particularly in places like Hawaii where levels of extraneous mater in cane are high. An effective dry cleaning system was designed in Hawaii (McEchloe and Lewis, 1974) and dry cleaning systems are used commercially in Cuba and Reunion.

To-day an added incentive for dry-cleaning is the fact that there is a demand for fibre so that if trash-laden cane is dry cleaned then revenue from the fibre could off-set the cost of cleaning. By dry cleaning unburnt untopped cane the fibre supply from unit area of land would be increased to 187% of that from BT cane.

Obviously dry cleaning would be futile if the cost of delivering the extra fibre to the factory was above the value of the fibre. The estimated costs of cutting, loading and transporting the additional fibre are as follows (all relative to BT cane):

	Unburnt topped	Burnt topped	Unburnt untopped	Burnt untopped
Cost of harvesting and transporting additional fibre (R/t <u>dry</u> basis)	17.2	-	23.7	15.6

These costs are well below the coal equivalent value of dry bagasse.

It might be argued that tops and trash are not suitable for use in many bagasse by-products such as paper. There is however the option of using the tops and trash as boiler fuel and thus releasing more genuine bagasse as surplus. The analyses of tops and trash (de Beer *et al.*, 1989) show that their weighted average moisture content (from unburnt untopped cane) would be about 51% and they could therefore be lightly knived or shredded and fed directly to boilers without requiring expensive dewatering. Alternatively, air separation by the cleaning plant may enable most of the wetter tops to be collected separately and fed to existing dewatering mills.

In an attempt to assess the economics of dry cleaning the computer model was used to simulate a cleaning system which would remove 75% of the tops and 80% of the trash from unburnt untopped cane. Profit from the cleaned cane was computed (with transport expenditure being that of uncleaned cane but no expenditure for cleaning included) and to this was added the value (coal equivalent) of fibre removed by the cleaning. This total profit was then compared with that which would accrue if the cane was processed without cleaning, and the difference in these profits was taken as the break-even cost of dry cleaning (Table 6). Sucrose losses caused by dry cleaning have not been taken into account. With burnt cane, McElhoe and Lewis (1974) measured cane losses of 0.55% but with unburnt cane the losses would be higher.

TABLE 6.--Break-even costs (SA Rands/t) for cleaning unburnt untopped cane.

Harvesting system to break-even with	Burnt Topped	Unburnt Topped	Burnt Untopped	Unburnt Untopped
Break-even costs of Cleaning unburnt untopped cane (R/t)	2.9	3.7	3.7	4.6

Judging from measurements of extraneous matter at South African Mills (Cargill, 1976) much of the cane delivered is effectively either burnt untopped or unburnt topped. The break-even cost of cleaning this will be less than for unburnt untopped cane because the effects of the cleaning would be less dramatic.

Non-quantified benefits of dry cleaning include: (a) reduced wear due to less soil and stones, (b) decreased need for burning of cane, with its attendant pollution problems and accelerated deterioration (see later), (c) shorter seasons or less capital equipment for equivalent sugar production.

One problem with dry cleaning at the factory in the South African industry is that cane samples for payment purposes are taken after the cane has passed through the factory preparation equipment whereas weighing is done at a vehicle weighbridge. The present hatch sampling system would have to be replaced by a system operating at the weighbridge.

Deterioration between field and factory

Following the development of a method for monitoring cane staleness (Lionnet and Pillay, 1988) it has been possible to use ethanol measurements in cane extracts as indicators of sucrose loss (de Robillard *et al.*, 1990). The losses are higher than had been anticipated and it is now realised that significant losses were being masked because they can occur without appreciably affecting sucrose % cane. The latter is elevated by moisture loss during deterioration.

When cane is burnt, deterioration is often serious because, for practical reasons, the area of cane burnt at any one time is often larger than can be harvested in the ensuing day and so delays between burning and crushing are prolonged. This is particularly serious after hot fires which cause cracking of the stalks thus allowing entry of microorganisms. Calculations of profit with different harvesting systems and delays suggest that it is often better to have fresh trash-laden cane than clean cane one day older (assuming that all the fibre is worth coal equivalent) (Table 7).

TABLE 7.--Relative profits with different harvesting methods as affected by time delays between harvesting (at 22°C) and crushing

Time delay (h)	Harvesting method			
	Unburnt topped	Burnt topped	Unburnt untopped	Burnt untopped
50	115	125	107	117
60	115	125	107	117
70	113	118	101	111
80	90	(100)	84	94
90	74	84	70	79
100	61	70	57	66

The results in Table 7 were generated by computer simulation. Real measurements of purity drops were made during the harvesting trials by comparing hand cleaned fresh stalks from the field with hand cleaned stalks collected on arrival at the factory (Table 8).

TABLE 8.--Clean cane purities in the field and at crush

Trial No.	Burnt	Fresh	Clean stalk purity	
			At crush	Difference
1 (UT)	No	91.5	90.8	0.7
2 (BT)	Yes	92.7	89.6	3.1
3 (UU)	No	90.9	90.9	0.0
4 (BU)	Yes	91.8	89.4	2.4

The high purity differences after burning support the suspicion that burning accelerates deterioration. When weighing up the sucrose losses associated with dry cleaning it is important to remember that the common alternative method of removing trash (ie burning) may sometimes cause greater losses.

CONCLUSIONS

After many years of research into sugar processing it is now apparent that the harvesting of cane is worthy of more research attention from those concerned with processing.

Detailed information is required before harvesting systems can be optimised for different circumstances, particularly now that fibre supply is becoming more important.

Measurements show that fibre supply can be substantially influenced by harvesting technique, to an extent much greater than is likely to be achieved by selection for high fibre cane varieties.

To incorporate fibre supply as a factor in the economic equation it is necessary to consider the economics not just from a grower or miller point of view but from a more holistic perspective, with the standing crop as the starting material. Soil conservation must also be considered.

The trend towards more fibre beneficiation through increased use of tops and trash means that research on colour removal at the factory assumes increasing importance, always remembering that one way to deal with colour is to keep it out of the factory.

Research on cane cleaning would be highly relevant to colour problems.

The conflicting requirements of high fibre supply and good cane quality can probably be economically addressed by the installation of efficient dry cleaning systems located at factories.

The many interacting factors which determine the most economic harvesting system for a particular set of circumstances make it imperative that a user-friendly computer model be developed for use by growers and millers. The model will be particularly valuable for assisting these parties to assess their mutual benefits in taking a joint approach to cane harvesting, and to develop appropriate payment systems in response to changing demands for fibre.

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DISCUSSION

Question: Congratulations on a very interesting paper. I agree completely with your statement that one way to deal with color is to keep it out of the factory. The problem is the same for beet processing. In Italy, at least, if we have a lot of beet tops (from incorrectly topped beet) coming into the factory, a lot of coloring matter enters the process stream. There will be low purities, high color, high inorganic non-sugars, etc. We must decide if it's better to receive the tops, which do contain a certain amount of sugar but also contain a lot of color and non-sugars, or to lose the sugar in the top.

Purchase: Yes, that is a similar problem.

Question: You mention that there is some work in progress on cellulose hydrolysis with subsequent production of single cell protein. You also produce a lot of molasses: I wonder if you are directing research toward single cell protein production from molasses.

Purchase: No, we are not investigating SCP production from molasses per se. With our increasing population, we are facing a growing demand for white meat. At the same time, one traditional feed for chickens, which is fish meal, is becoming scarce and expensive. An alternative to fish meal is single cell protein. We have reached the point where we have a pilot plant for this product. Predictions are that in about 10 years time we will have a demand for some 100,000 tonnes of single cell protein, for chicken feed. That production will require some 200,000 tonnes (dry weight) of bagasse.

Question: Do you make fiberboard from your bagasse?

Purchase: On a small scale.

Question: On an economic basis which bagasse products have the greatest return, or potential return?

Purchase: At the moment the single cell protein is still in the speculative stage. We don't have enough real information to be convinced that it is the answer to the problem. So at this time, there is no answer to that comparison.

Question: I certainly compliment you on the depth you've gone into in this very interesting opportunity.

Question: What about sugar losses going out in the extra bagasse and molasses?

Purchase: This is the main reason that we see a need to get the process balance correct, so that we don't lose the sugar gained from increased extraction to bigger losses to molasses. In our computer modelling calculations, we do take that possibility into account. I'm not convinced that they're all that accurate, but we do have formulae which enable us to predict molasses production with different qualities of cane. The loss is one reason why we see a need for dry cleaning of cane. Traditionally, dry cleaning has been to improve cane quality. We now have an additional reason to look at dry cleaning - to improve the fibre sugar yield without increasing sugar losses in molasses and bagasse.

Question: Congratulations on an excellent paper, and a mammoth project. On the influence of ash: some work that we carried out some years ago indicated a very significantly higher figure for ash % impurity in tops. I wonder if you'd like to comment on the influence of ash on overall recovery.

Purchase: I can confirm that our results also show a tremendous increase in ash associated with trash and tops. A lot of that ash is in fact insoluble ash: sand and grit. We have taken it into account in calculating our predicted molasses purities, etc. Again - its another reason to look at dry cleaning. If there is sand in the tops and trash, we can remove some of that sand by dry cleaning.

STARCH: PROCESS PROBLEMS AND ANALYTICAL DEVELOPMENTS

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INTRODUCTION

The presence of starch in sugar processing and the problems it engenders have been written about for many years (Feuilherade, 1929). High starch levels in cane periodically appear and have given problems over the years in Australia (Hogarth, 1968), Louisiana (Balch, 1953; Cashen and Friloux, 1966) and South Africa (Alexander, 1954; Alexander and Matic, 1974), often in areas characterized by temperate or subtropical climate.

Biologically, both starch and sucrose act as major carbohydrate storage sinks--most plants store starch and only small amounts of sucrose, but a few plants, such as sugarcane and sugarbeet, store sucrose as the major energy reserve. In sucrose-storing plants such as the sugarcane, very little starch is ever produced (typically less than 1 percent of total solids); nevertheless, there is always a small amount of starch present throughout the tissues of the cane plant, especially in the immature growing point, the green leaves and nodes. This small pool of starch presumably acts as an alternate energy storage form and fluctuates diurnally. During processing of the cane stalks, the starch is expressed into the juice.

In the previous two years, starch levels in Louisiana and Hawaiian raw sugars have occasionally reached levels high enough to adversely affect their performance in the refinery. As a result, a program was instituted at SPRI to analyze starch and to observe its behavior in mills and refineries.

Review of Starch Methods

Methods for starch analysis fall into two general categories: (1) colorimetric, based on the starch-iodine complex; and (2) methods that isolate the starch, hydrolyze it and measure the glucose produced. A few older methods rely on gravimetry; i.e., precipitation of the starch and weighing the resulting purified precipitate.

The most common methods of starch analysis are colorimetric methods that utilize the starch-iodine complex, usually the blue amylose-iodine complex, with absorption maximum in the range of 600-650 nm. Less usual, but sometimes also used is the violet/brown amylopectin-iodine complex, in the absorption range of

540-570 nm, as reported by Charles (1968) to examine starch in char-filtered and granulated products.

Many variations of the colorimetric method using iodide/iodate exist. These include different ways to precipitate the starch or, alternately, no precipitation; different ways to dissolve the starch; use of different standard starches; different wavelengths of determination.

In a like manner, many variations of the second category of method exist, including starch precipitation and purification procedures, in the way the starch is hydrolyzed (enzymatically or by different acids for varying times) and especially in the way the produced glucose is measured -- polarimetrically, titrimetrically, colorimetrically, chromatographically. These methods have not found widespread use in the sugar industry, whereas the more convenient colorimetric methods based on iodine have.

No official method for starch analysis in sugar exists, although ICUMSA (International Commission for Uniform Methods of Sugar Analysis) has repeatedly observed the need for a method. The SMRI method (Alexander, 1954; Matic, 1971), developed as a modification of the original method by Balch (Balch, 1953), has received the most attention in this regard. In 1974, the ICUMSA referee for polysaccharides recommended its adoption as a tentative method. However, without the benefit of a collaborative test, the recommendation was not accepted (ICUMSA, 1974).

MATERIALS AND METHODS

The SMRI method, as detailed in the Cane Sugar Handbook (Meade & Chen, 1977; Chen, 1985), served as the starting point for starch analysis. Because of difficulties with the paper filtration method, alternate filtration methods were tested; the use of a sintered glass filter showed the best results.

The modified method is detailed in Appendix I. The modifications included:

- 1) Specification of Celite analytical grade filter aid for filtration.
- 2) Shortening the one hour settling time (starch precipitation step) to 15 min.
- 3) Use of 60 ml fritted glass funnels for isolation of precipitated starch, instead of Whatman No. 5 filter paper.
- 4) Use of less filter aid and no correction for volume of filter aid.

- 5) Instruction to boil calcium chloride solution gently instead of vigorously.

Process samples were obtained from four refineries and three mills to examine the behavior of starch in process. The starch in raw sugars from several sources as well as that in white sugars was also surveyed. For raw sugarcane juices, samples taken from the mills in Louisiana were immediately frozen and when defrosted, aliquots were precipitated immediately with ethanol to prevent degradation of the starch by native amylase enzyme. The raw juices from Florida were precipitated with alcohol immediately upon collection and transported to New Orleans under refrigeration.

RESULTS AND DISCUSSION

It has been well established (Matic, 1971; Vignes, 1974; Stevenson and Whayman, 1978) that a major source of variation in any colorimetric method based on the starch-iodine complex is the source of starch used for the calibration curve. This effect is shown in Table 1. Also shown is the effect of not using fresh reagents. Using filter paper resulted in shallower slopes (i.e., less color in sample), and this was found to be due to some adherence of starch on the filter paper and loss of starch under the paper. It was for this reason that the method was changed to use sintered glass funnels, which are used in other polysaccharide methods used at SPRI (Roberts, 1980). For the purpose of this report, the calibration curve obtained using Fisher certified soluble starch was used.

Table 1. Effect of varying parameters on slope of starch calibration

Type of Starch	Slope (Glass funnels)	Slope (Filter paper)
Baker analyzed	0.216	0.158
Fisher certified soluble	0.269	-----
Cream corn starch	0.388	-----

	Reagents fresh	Reagents not fresh
Cream corn starch	0.388	0.187

The repeatability of the method was tested at three concentrations, shown in Table 2. As would be expected, the variability increases as the concentration decreases. We have also found that attempts to store the precipitated starch in the filter aid (as when there is no time to finish the analysis that day), results in decreases ranging from 8-20%. A high level of color in the sample does not interfere in this analysis since much of the color is washed out of the precipitate and the very small remaining amount has little absorbance at 600 nm. We have found that the maximum amount of error introduced by color is on the order of 20 ppm starch, and this occurs in molasses and remelt liquors with starch levels usually well over 500 ppm.

Table 2. Variability in starch-iodine method used at SPRI

	Sample A	Sample B	Sample C
	442	321	22
	448	325	19
	430	319	23
	455	308	20
Mean	444	318	21.0
S.D.	10.6	7.3	1.8
CV (%)	2.4	2.3	8.6

Recently, Tsang (1990) reported an I.C. (ion chromatography) method for determining starch, which measures the glucose produced after enzymatic hydrolysis of starch. His results provided good confirmation of the results obtained using the iodine method described in this report. The comparison of the two methods is shown in Table 3. The iodine method averaged about 17.5 ppm lower starch than the I.C. method.

Table 3. Comparison of iodine method and IC method for starch determination in raw sugars.

Raw Sugar	ppm Starch		Difference (Iodine-I.C.)
	Starch by Iodine	Starch by I.C.	
A	117	116	+ 1
B	163	229	-66
C	193	181	+12
D	227	282	-55
E	248	229, 228	+19, +20
F	262	255, 294	+ 7, -32
G	415	414	+ 1
H	453	492	-39
I	459	464, 475	- 5, -16
J	498	514	-16
K	499	532	-33
L	519	580	-61

Starch in Mills

Tables 4 and 5 show the starch levels in two Louisiana mills and Table 6 shows the same for a Florida mill. The very high levels of starch in Mill A (Table 4) may be due, in part, to the fact that the cane was being harvested during wet conditions, and the cane in the yard was muddy with a lot of adhering leaves and trash. This mill was adding heat stable enzyme at the evaporator; it also has a syrup clarification system. The enzyme caused a 42% decrease in starch. Neither the juice nor syrup clarification steps decreased the starch level.

Table 4. Starch in Louisiana Mill A

Sample	Starch, ppm on solids
Crusher juice	1994
Dilute (mixed juice)	1643
Residual juice	1158
Clarified juice	1255
Evaporator syrup (enzyme added)	731
Clarified syrup	770
Filtered syrup, #1	748
Filtered syrup, #2	731
A raw	278
B raw	576

Table 5. Starch in Louisiana Mill B

Sample	Starch, ppm on solids
Crusher juice	609
Dilute (mixed) juice	600
Last mill juice	905
Clarified juice	464
Syrup	904
A sugar	203
B sugar	359
C massecuite	920

Table 6. Starch in Florida Mill C

Sample	Starch, ppm on solids	
	1/29/90	1/30/90
Crusher juice, Mill E	127	355
Crusher juice, Mill W	284	242
Last roll, Mill E	100	261
Last roll, Mill W	402	327
Dilute (mixed) juice, Mill E	111	314
Dilute (mixed) juice, Mill W	249	214
Clarified juice	253	269
Syrup	272	229
A sugar	62	59
B sugar	85	80

The starch in the Florida mill is considerably lower throughout than in either of the Louisiana factories and is reflected in the raw sugars produced with less than 100 ppm starch.

Soluble and Insoluble Starch

In the living cane plant, starch occurs naturally in small (1-5 μ), spherical granules (Stevenson and Whayman, 1976; Vignes, 1974). These well-organized structures are insoluble and easily removed by filtration, centrifugation and settling. The granules are expressed into the juice during milling and are subsequently solubilized (gelatinized) by the action of heat and lime.

Starch in its different forms will behave differently in process: The large granules (5 μ) will gelatinize during process and increase viscosity. Small, intractable granules (1 μ), as reported by Parrish, et al., (1984) will not gelatinize but rather will plug the pores of the filter medium and block filters. Large, intact starch granules will have little effect on filtration until they are gelatinized.

The presence of soluble compared to insoluble starch was studied by two methods. In the mill, samples of cane juice were analyzed before and after filtering through Reeve-Angel sugar paper. The starch remaining in the sample after filtration was assumed to be soluble. In raw sugars, the method of analysis was altered to omit the gelatinization step -- boiling in 40% calcium chloride solution. The sample was suspended in the calcium chloride but no heat was applied. The results from this "cold" method were assumed to reflect the soluble starch.

Results for the mills are shown in Table 7. The data indicate that starch is progressively solubilized across the mill and a significant proportion of the total starch is already gelatinized coming off the mill. The data for Mill B also point out that relatively more starch may be contributed during maceration. The inclusion of large amounts of impurities relative to sucrose has been shown for many other constituents in maceration.

Table 7. The presence of soluble starch in mills

	ppm Starch/solids		
	Total	Soluble	%Soluble
Mill A			
Crusher juice	1994	232	11.6
Residual juice	1158	1032	89.1
Dilute (mixed) juice	1643	661	40.2
Mill B			
Crusher juice	609	494	81.1
Last mill juice	905	905	100.0
Dilute (mixed) juice	600	486	81.0

The results for 30 raw sugars using the "cold" method for soluble starch showed that an average of 70.4% of the starch in the samples was soluble. This ranged from a low of 18.3% to a high of 95.9% soluble starch. Figure 1 shows the scatter diagram for 18 of the samples. The presence of starch in different proportions of soluble vs insoluble could certainly affect filtration tests that are done on room temperature solutions, and it would probably be a good practice to boil and then cool a raw sugar prior to performing any filterability tests.

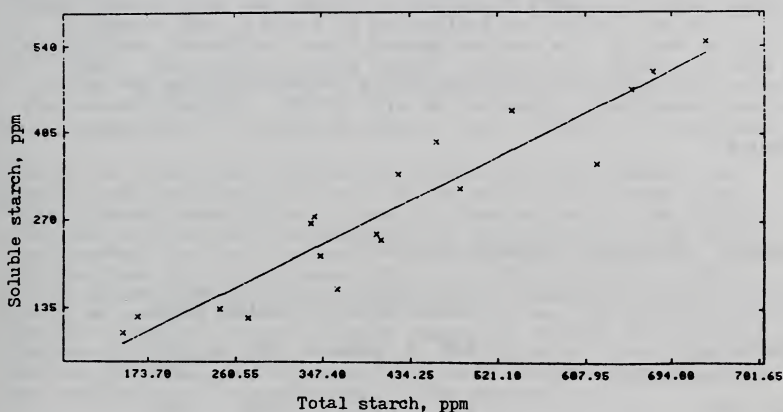


Figure 1. Scatter diagram showing amount of soluble starch versus total starch in 18 raw sugars

Starch in Refineries

Table 8 shows the concentration of starch in four refineries. Removal of starch during affination ranged from 2.4% (Ref. D) to 43.9% (Ref. A), and averaging 24% removal. This compares to the average of 30% removal of total polysaccharides on affination found in a previous study (Godshall, et al., 1987), and indicates that, as with other polysaccharides, starch is preferentially distributed inside the raw sugar crystal.

In all four refineries, the recycling of impurities arising from the use of remelt and/or wash waters resulted in increased starch in melt liquor relative to affined sugar. Clarification (Ref. A uses carbonatation; others use phosphatation) removed 33-64% of the starch in melt liquor. It is noteworthy that, since recycling of impurities negated the good effects of affination, clarification was essentially the first significant step in starch removal. These rates of removal are somewhat higher than the 25-35% removal reported for laboratory phosphatation (Murray, et al., 1976), and may reflect the effect of flocculants.

Decolorization methods (either char, resin, or a combination), in general, did not remove significant starch, the percentages being 0.01, 0.06 and 0.005% for Refineries A, B and C, and a high of 17.8% for Ref. D. In another sample from a different char filter in Ref. C (result not shown), starch removal was 61.5%. Other studies have shown that absorption of polysaccharides can occur on fresh adsorbents (Roberts, et al., 1978). Crystallization was by far the most effective way to remove starch in this set of samples.

Table 8. Starch in refinery processes

Sample	ppm starch on solids basis			
	Ref. A	Ref. B	Ref. C	Ref. D
Raw Sugar	228	101	259	167
Washed Raw	128	64	225	163
Melt Liquor	228	89	288	187
Clarified Liquor	114	32	192	101
Decolorized Liquor	113	30	191	83
Refined Sugar, No. 1	22	21	20	NA

NA = Sample not available

Like other polysaccharides, starch rapidly builds up in successive white sugar strikes. This is shown in Table 9.

Table 9. Distribution of starch on successive boilings in a refinery

Sample	ppm Starch
Decolorized liquor	191
No. 1 sugar	20
1st syrup	266
No. 2 sugar	68
2nd syrup	615
No. 3 sugar	206
3rd syrup	915
No. 4 sugar	494
4th syrup	1970

CONCLUSIONS

One of the unresolved problems in starch analysis remains the question of what starch to use for the calibration curve. As Table 1 shows (also shown by many previous workers), the source of starch will affect the magnitude of the results -- the shallower curves (ie, small slope) will give higher results of starch in sugar than will the steeper curves. Before this or another method can be widely adopted by the sugar industry, agreement should be reached on what type of starch to use for calibration. Barring a reliable source of pure sugarcane starch, any starch from another source will provide an estimation of the starch present, but the same type should be used consistently to ensure that results will be comparable to one another.

Starch results obtained using various starch standards can be compared to a method such as the new I.C. method recently reported by Tsang, et al. (1990), which has the potential to provide an accurate estimation of the actual starch. In this manner, a correction could be applied to obtain absolute starch, or a starch standard that most closely approximated the absolute value could be specified.

The results reported here have shown how starch behaves in process. In most respects, starch behaves like other polysaccharides. A major difference noted is a greater tendency to be removed during clarification, probably as a result of the use of efficient flocculants and because the starch is not all solubilized at this state. Starch appears to have greater tendency to be included inside the raw sugar crystal than in the white sugar crystal, but the sample size is too small to draw a conclusion. Changes in the amylose-amylopectin ratio during refining have been suggested (Charles, 1968; Murray, et al., 1976) as the cause

for variable behavior in process, and may also account for the fact that crystallization does not show uniform elimination of starch. Addition of amylase enzyme in the mill offers a solution to extremely high starch levels which may occasionally appear.

ACKNOWLEDGEMENTS

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APPENDIX I. METHOD FOR STARCH ANALYSIS USED AT SPRI

Scope and Principle

Starch is precipitated by ethyl alcohol, caught on a mat of filter aid, solubilized by boiling in calcium chloride solution, reacted with iodine and colorimetrically analyzed as the blue starch-iodine complex at 600 nm.

The linear range of analysis is 0 to 500 ppm, but this can be extended by diluting the final solution used for color development. The test is applicable to raw and refined sugar, juices, and process syrups and liquors.

Equipment

Spectrophotometer
Stirring and heating plate
Magnetic stirring bars
Analytical balance
pH meter

Supplies

1 cm cuvettes for spectrophotometer--glass, quartz,
or polystyrene
Analytical grade filter aid (Celite C-211)
60 ml coarse fritted funnel
Pre-pleated filter paper (Schleicher & Schuell #588)
Filter funnel large enough to hold pleated filter paper
Beakers (250 ml)
Filter flasks
100-ml volumetric flasks
50-ml volumetric flasks
Watch glass large enough to cover 250-ml beaker
Pipets, 0.5, 2.5, 5, 10 and 20 ml
Additional pipets with capacity of 15, 25 and 30 ml
for calibration curve
Graduated cylinders, 100 ml, 500 ml
Beaker (100 ml and 1 L) - for calibration curve only
500-ml volumetric flask - for calibration curve only
Large rubber bulb

Reagents

Deionized water

Absolute ethanol

80% ethanol (80 ml ethanol + 20 ml water)

40% calcium chloride adjusted to pH 3 with 2N acetic acid

2N acetic acid (120 g glacial acetic acid/liter)

10% potassium iodide, freshly prepared daily (10g KI/100 ml)

M/600 potassium iodate (0.03567 g KIO₃/100 ml) or (0.3567 g/L)

(Reagent is stable for one week)

Pure starch for iodometry

Refined white sugar with as low starch content as possible,
preferably beet sugar if available.

The Calibration Curve.

1. Determine the moisture content of the starch by drying 10 grams overnight in a 95-100C oven. The difference in weight between "wet" and dried starch divided by the original wet weight of starch times 100% is the moisture content:

$$\% \text{ moisture in starch} = \frac{\text{Wet wt} - \text{dry wt}}{\text{Wet wt}} \times 100\%$$

Discard the dried starch after determining the moisture content. For the calibration curve, use only unheated starch and adjust weighed amount for the calculated moisture content. (See No. 2)

2. Determine how much starch is needed to obtain the equivalent of 500 mg dry starch: For example, if the moisture content was 10.5%, 500 mg dry starch = $500 / (1.00 - 0.105) = 558.66$ mg starch.
3. Make a stock solution of starch to contain 1 mg starch/ml:
 - a. 500 mg dry starch equivalent (see No. 2 above) is slurried with about 10 ml water in a 100-ml beaker.
 - b. This is quantitatively added to 300 ml boiling water in a 1 L beaker, and boiling is continued for 1 min.
 - c. Cool the solution.
 - d. Transfer to a 500 ml flask and adjust to volume with water.

4. The solutions listed in Table 1 are prepared in 250-ml beakers.
5. Add 1 gram filter aid to each sample in the beaker.
6. To each solution, add 100 ml absolute alcohol.
7. Allow the solutions to stand at least 15 min.
8. Make a filter aid mat: Place 1.00 g filter aid into a 100 ml beaker and add approx. 50 ml water to make a slurry. Pour into a 60 ml coarse fritted funnel, pulling a gentle vacuum to remove the water and make a mat of filter aid on the funnel. Do not allow the mat to become so dry that it cracks.
9. Quantitatively transfer each solution to a separate funnel; use 80% ethanol in a squeeze bottle to wash out all traces of solution and filter aid in the beaker into the funnel.
10. Filter each solution; wash twice with 25 ml 80% alcohol.
11. Quantitatively transfer the filter cake into a 250-ml beaker. (See Figure 1 on how this is done.)
12. Add 40 ml of acidified 40% CaCl_2 to the filter cake and boil gently for 15 min. (Cover the beaker with a watch glass to prevent excessive evaporation.)
13. Allow sample to cool.
14. Transfer cooled sample to a 100 ml volumetric flask and make to volume.
15. Transfer cooled sample to a 10 ml volumetric flask and make to volume.
16. Pipet 10 ml of filtrate into a 50 ml flask.
17. Add, in the following order, swirling to mix after each addition:
 - a) 10 ml water
 - b) 2.5 ml 2N acetic acid

- c) 0.5 ml 10% KI
- d) 5.0 ml M/600 KIO₃
- e) bring to volume with water

18. Mix contents thoroughly. Allow color to develop 2 min.
19. Blank the spectrophotometer using solution 1 (only sugar with no starch added).
20. Determine absorbance at 600 nm of each of the standard solutions in a 1-cm cell.
21. The calibration graph is prepared by plotting the absorbance vs. mg starch per 50 ml.

A calibration curve should be prepared at least once a year or whenever the conditions of analysis change, such as a new source of starch or other reagents, new spectrophotometer, different cuvette material, or if the spectrophotometer is moved or the light source is changed. A new curve should also be prepared by each new analyst for practice and comparison.

Table 1. Starch Solutions Used for Calibration Curve

Solution No	Refined sugar (g)	Water (ml)	Starch solution (ml) (mg starch)	Final starch concentration at color level (mg/50 ml)
1	25	30	0	0.0
2	25	25	5	0.5
3	25	20	10	1.0
4	25	15	15	1.5
5	25	10	20	2.0
6	25	5	25	2.5

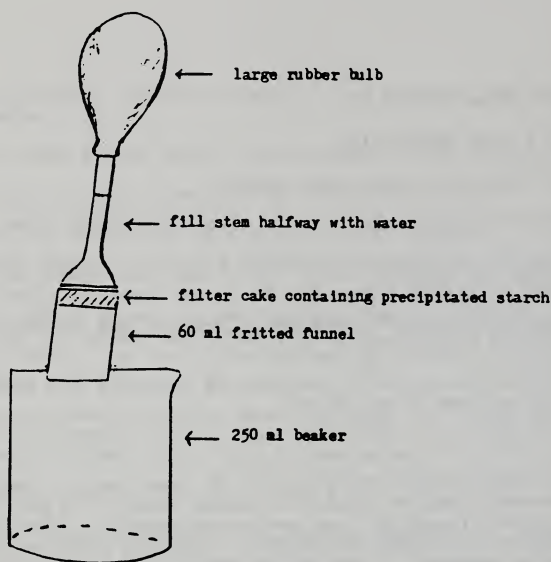


Figure 1. Demonstration on how to remove filter cake from fritted funnel.

Instructions: Invert the funnel containing the filter cake and fill the funnel stem halfway with water. Position the funnel over a clean 250-ml beaker, attach the rubber bulb to the end of the stem and press. The pressure will eject the cake. Repeat, if necessary. Wash the inside walls of the funnel with a minimum of water. (This adds about 30-35 ml of water to the solution.)

PROCEDURE FOR DETERMINING STARCH IN CRYSTALLINE SUGARS

1. Weigh 25.0 g sugar into a 250-ml beaker.
2. Add 30 ml water with a graduated cylinder.
3. Add 1.00 g filter aid to sample; stir to dissolve sugar.
4. Add 100 ml absolute alcohol to contents of beaker and allow to stand at least 15 min.

5. Add a slurry of 1.00 g filter aid to a 60 ml coarse fritted funnel; pull a vacuum to remove water and make a mat; do not allow to get so dry that the mat cracks.
6. Quantitatively transfer the contents of the beaker into the funnel, using 80% ethanol to remove the last traces of solution from the beaker.
7. Filter the solution and wash twice with 25 ml 80% ethanol.
8. Quantitatively transfer the filter cake into a 250-ml beaker (Figure 1), and add 40 ml acidified 40% CaCl_2 . Boil gently for 15 min.
9. Cool and transfer to a 100 ml volumetric flask and make to volume. Mix thoroughly.
10. Filter the contents of the flask through a pre-pleated filter paper, discarding the first 40 ml of the solution.
11. Pipet 20 ml of filtrate into a 50-ml volumetric flask, and add, in order:
 - a) 10 ml water
 - b) 2.5 ml 2N acetic acid
 - c) 0.5 ml 10% KI
 - d) 5.0 ml M/600 KIO_3
 - e) bring to volume with water
 - f) mix thoroughly
12. Prepare a reagent blank as follows: 30 ml water in a 50-ml volumetric flask plus parts b) through e) above.
13. After at least 2 min, read the absorbance of the samples at 600 nm, after blanking the spectrophotometer against the reagent blank.
14. The ppm starch in sugar equals milligrams of starch (from the calibration graph) times 200.

Note that for sugar analysis, 20 ml of filtrate is taken for color development, while for the calibration curve, only 10 ml of filtrate is taken.

The calculation for determining ppm starch in crystalline sugar is developed in the example below:

Assume 0.5 mg starch/50 ml from the calibration curve:

$$\text{ppm} = \frac{B}{A} \times \frac{D}{C} \times E \times F$$

A = Grams sugar taken for analysis	= 25 g
B = Vol the gelatinized starch and filter aid are in after boiling with calcium chloride	= 100 ml
C = Aliquot taken for final analysis	= 20 ml
D = Total solution for color development	= 50 ml
E = Starch from calibration curve	= 0.5 mg/50 ml
F = Factor to convert results to ppm	= 1000 $\mu\text{g}/\text{mg}$

$$\text{ppm} = \frac{100 \text{ ml}}{25 \text{ g}} \times \frac{50 \text{ ml}}{20 \text{ ml}} \times \frac{0.5 \text{ mg}}{50 \text{ ml}} \times \frac{1000 \mu\text{g}}{\text{mg}} =$$

$$200 \left(\frac{\mu\text{g starch}}{\text{g sugar}} \right) \times 0.5 = 100 \text{ ppm}$$

If the absorbance is too high (>0.8), it is possible to dilute the sample by putting less sample into the 50 ml flask. Start off with a 2:1 dilution (10 ml instead of 20 ml) and repeat the color analysis. The calculated ppm starch will then have to be multiplied by the dilution factor, which is 2 in this case, eg:

$$\text{Dilution factor} = \frac{20 \text{ ml}}{10 \text{ ml}} = 2$$

PROCEDURE FOR DETERMINING STARCH IN JUICES (Proposed)

The sample should be analyzed as soon as possible after obtaining the juice; if sample has been frozen, analyze immediately after defrosting. Do not filter the sample prior to analysis, as much as the starch will be lost.

Sample preparation:

1. Determine the brix of the juice.
2. Weigh 25 g cane juice into a beaker, add 5 ml water and 1.00 gram filter aid. Stir to mix thoroughly.
3. Add 100 ml absolute ethanol to precipitate starch for at least 15 min.
4. Proceed as for crystalline sugar, starting with step 8.

Calculation:

200 x mg starch for the calibration graph will give the ppm starch in the total juice (25 g). Dividing the calculated ppm starch by the brix will give the ppm starch on a juice solids basis.

Example: ppm starch in juice from curve = 200 ppm
juice brix = 17.3

$$\text{ppm starch in juice solids} = \frac{200}{.173} = 1156$$

PROCEDURE FOR DETERMINING STARCH IN PROCESS SYRUPS, LIQUORS AND MOLASSES (Proposed)

Sample preparation (for samples ≥ 60 Brix):

1. Determine the brix of the syrup.
2. Weigh 25 g syrup into a 250-ml beaker, add 20 ml water and 1.00 g filter aid; stir to dissolve.
3. Add 100 ml absolute ethanol and let stand 15 min.
4. Proceed as for crystalline sugar, starting with step 8.

Calculation:

The calculation for ppm starch is the same as for juices, above.

DISCUSSION

Question: In your discussion of your analytical procedure you listed three standard starches that were used for calibration purposes. Were those all corn starches?

Godshall: One of them, the one with the highest slope, was corn starch. The other two don't say on the labels, but we called their manufacturers who said that they are potato starch.

Question: I think that in corn starch you have the ratio of amylose to amylopectin that most closely approximates that in cane.

Godshall: Yes, The corn starch that we had was not certified, however. It is very hard to find corn starch that is certified by the manufacturer. That's why we did not use it, even though you would expect that corn, being a graminaceous plant, would have a more similar starch to cane than potato starch.

Question: You mention that much of the starch is removed by carbonation. Do you know approximately what percent?

Godshall: At high starch levels in raw sugar, about 50 to 60% is removed.

Question: I think that the amount of starch you can find in the crystal of sucrose is related to the rate of crystallization. Have you ever tried to crystallize very slowly in the presence of high amounts of starch?

Godshall: No, we have not done that.

CHARACTERIZATION OF SOFT SUGARS

Richard Riffer

California and Hawaiian Sugar Company

INTRODUCTION

The complexity of multi-component physical systems such as soft sugars does not permit exhaustive characterization. Whereas granulated sugar is essentially a single substance, nearly 100% pure sucrose, soft sugars contain hundreds of non-sugar components in variable concentrations, from very minute parts per billion (certain flavorants) to several percent (water). Even trace constituents can influence product quality, particularly sensory properties. For example, furaneol, a contributor to caramel aroma, can be detected by sensitive individuals at a level of 40 parts per trillion (SPRI Technical Report No. 2).

An important visual property of soft sugars is color, which results principally from substances produced at low concentration during processing and to only a small degree from naturally occurring plant pigments. The colorant fraction can be highly heterogeneous: because of differences encountered in raw juice composition as a result of genetic and environmental factors, and because of almost limitless possibilities for variations in color-forming reactions during sugar manufacture, it is likely that no two colorant fractions are exactly alike. Moreover, because no definite or constant composition can be expected for most of the colorants, usual criteria of purity cannot be applied to these substances (Riffer, 1988).

In order to minimize this inherent heterogeneity, it is advantageous to obtain raw cargoes from a single source or geographical area, so that their quality is consistent. Constancy of refining technique also helps to assure uniformity in the colorant fraction.

Virtually every decision made in operating a refinery affects the properties of the soft sugar ultimately produced, from the procedure used to wash a spent bone char cistern to that for calibration of a process pH meter. Of course, in most cases the influence of such factors is slight, but with so many variables to consider, it is sometimes difficult to pinpoint the source of variation in a measurable attribute. Experience has proven to be our most valuable resource for ensuring uniformly high quality soft sugars. The expertise of knowledgeable individuals, accumulated from many years of experience, has provided a body of valuable information about the complex chemistry of soft sugars.

Soft sugars are valued and used because of their distinctive physical, chemical, and organoleptic attributes. This article describes the basis of these characteristics and the means for their measurement.

SENSORY PROPERTIES

Visual

Several systems are used to communicate or differentiate colors precisely. The two most common are the L^*a^*b and Munsell notations, which are interconvertible. These systems incorporate brightness and saturation components with a hue term to describe a color. The methods are at present used for such applications as color control in the paint, printing, and textile industries, where the manufactured product is a surface. However, for granular substances, such as many foods, where diffuse reflectance is an important contributor to product appearance, reflectance measurement is a common means of color control. This is the case for soft sugar manufacture.

Solution color

Standard methods have evolved based on the premise that an instrumental measurement of color is most useful if it bears some relation to the color observed by the human eye. The most important limitation imposed by this consideration is the obvious one that measurement be made in the visible region of the spectrum. Although this restriction may appear self-evident, it has sometimes been recommended that measurement be made in the ultraviolet region of 270-280 nm, where many colorants display absorption maxima.

After many years of controversy, the U.S. National Committee on Sugar Analysis (USNC) in 1978 adopted a standard color measurement at 420 nm for all sugar solutions. The advantage of this wavelength is that it is near the maximum in sensitivity obtainable in the visible region. This is the wavelength used at C&H for all color measurement.

Non-standard methods continue to be used elsewhere, particularly for dark solutions, such as soft sugars or their components, or for certain raw sugar contracts. Typically readings are made at 560 nm. However, such measurements are of the same absorption seen at 420 nm, but at a region of lower sensitivity. Use of the longer wavelength is sometimes justified on the grounds that the 420 nm reading can be off scale for dark solutions, except under high dilution that could introduce a large error. However, recent models of spectrophotometers can accommodate high absorbancies, so this argument is not entirely valid.

Readings at 720 nm are sometimes made to determine the light scattering component of the attenuation index, but there is no theoretical basis for doing so. The rationale for this practice appears to be that absorption is so low in this wavelength region that interference with scattering is minimized. The haze method for dextran, which has ICUMSA status, stipulates 720 nm measurement, presumably on the basis of such rationale. However, it must be borne in mind that turbidity depends upon the refractive index gradient between medium and scattering species, not the concentration of that species: diluting the sample with extra pure sugar of the same refractive index does not change the turbidity. This is, of course, contrary to the "common sense" expectation. This does not mean that there is no relation between the concentration of scattering species and turbidity, but rather that scatter does not obey the Lambert-Beer law.

We have found that the best way to determine turbidity is by nephelometry, that is, measurement of 90° scatter. For routine monitoring of product quality, we filter samples before color measurement through 1.2 μ membranes, with the recognition that this procedure removes suspended solids but not colloidal matter.

Solution color measurement is made at pH 7.0, the USNC standard and the target pH for most sugar processing. This pH is not necessarily optimal, because (a) sucrose is actually most stable closer to pH 8; and (b) color measurement has a high pH dependence. A curve of color vs. pH displays a large derivative near pH 7, with an inflection point near pH 8.5 at 420 nm. On the other hand, colors measured in the pH range of 10.5 to 12 are insensitive to small pH changes but relatively unstable with respect to time. The degradation of absorbancy at high pH may not be noticeable if insensitive instrumentation is used.

Failure to adjust pH can not only result in deceptively light colors but can also suggest that impossible events have occurred, such as color removal during an evaporation step, or spontaneous decolorization that accompanies chemical or microbiological degradation.

According to the Lambert-Beer law, used for all colorimetry, the absorbancy index (or attenuation index) is inversely proportional to the concentration of colorant. Ordinarily one does not know the concentration of colored material in a sugar solution, so the indices are defined on the basis of the concentration of total solids (sugars plus non-sugars), a figure readily available from a RDS measurement.

Reflectance color

Although the color specification for most refined products is a solution or absorbance color, for soft sugars it is commonly a reflectance color, measured on the sample in the solid state.

A distinction should be made between specular reflection and diffuse reflection (Fig. 1). Specular means "without change in direction" and refers to light reflected from a smooth or polished surface, or from the first surface of contact. The meaning of this last concept will be described below. The light is reflected in a particular direction, and measurement indicates a position intermediate below zero gloss (matte) and a perfect mirror. The angle of incidence and that of detection are equal. Specular reflection can be correlated with absorbance data via the Kramer-Kronig transformation (Wihlborg, 1989), so it is theoretically possible to make such measurements using Fourier Transform infrared spectroscopy.

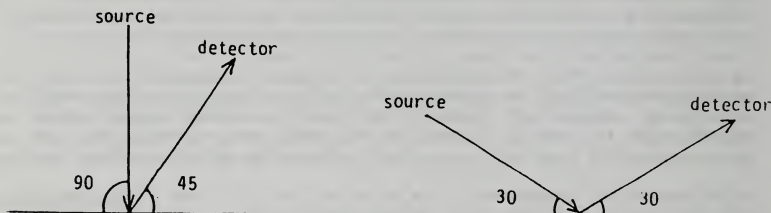


Fig. 1. Diffuse (left) and specular (right) reflectance.

Diffuse reflection is that from a matte surface. The reflected light travels in all directions, so the intensity or brightness is the same from every angle of observation; that is, it is isotropic. Detection is commonly as a function of wavelength, so sample color plays a role. In some measurement systems, specular components are rejected by subtracting photons detected at an angle equal to that of incidence. Here again, FT-IR systems may eventually replace traditional reflectance spectrophotometers.

Reflectance measurement of soft sugars is primarily of the diffuse type, with standardization using neutral shades-of-gray disks. The sample is illuminated at a low angle of incidence (0° = perpendicular) in order to obtain average characteristics unaffected by particle size and geometry. Under such circumstances, irregularities and voids are less likely to produce shadows. However, the optimum angle depends on the type of surface and must be determined experimentally.

In general, large angles, nearing grazing (90°), are best for low gloss and textured surfaces, such as textiles. High gloss surfaces, on the other hand, are best judged at low angles of

incidence and detection. For soft sugars, a red filter is commonly used ahead of the detector, because the result seems to correlate well with a reddish-brown or golden brown product that is pleasing to the eye. Samples prepared for reflectance measurement must obviously be sufficiently thick that readings are invariant to further increases in sample pathlength; that is, they must behave as though they were infinitely thick.

The instrument used for reflectance measurement at C&H is the Agtron (Reno, NV) M-500-A. Although our product specifications are based on direct Agtron readings, we additionally compute an Agtron-based "reflectance color," to provide continuity of data with a pre-Agtron system.

Instrument design is of importance, since surface properties depend on the light source: a small concentrated source gives a glossier appearance, whereas a large diffuse source makes the product look duller and more opaque. Consequently, reflectance data made on different instruments are not readily correlated. Detector geometry is important to sensitivity, because diffusely transmitted radiation is distributed according to the Lambert cosine law, $I = I_0 \cos X$, where X is the angle of detection from the normal.

Some measurement systems use full-spectrum analysis, determining a position on a red-green axis as well as on a yellow-blue one, but this can yield a complex configuration not easily correlated to visual properties. Since sugar liquors strongly absorb blue light, attempts to impose a blue reference may indicate a misguided effort to adapt color standards appropriate for other manufactured products, such as textiles or paper, to refined sugar products.

Specifying both a reflectance and an absorbance color for a product can lead to difficulties, because the two systems are only partially interdependent and correlate imperfectly. As an illustration of how the two can be at odds with one another, consider an over-heated product. Thermal degradation will result in a somewhat darker solution color, but the accompanying moisture loss would tend to give rise to a lighter reflectance color.

In a real system, such as a semi-translucent soft sugar sample, reflectance patterns are highly complex. When a sample is irradiated with a beam of photons, not all will interact identically. They will differ in penetration depth and in the number of scattering events. Incident light will partially penetrate the semi-transparent syrup film to the more opaque crystal interior; reflection, refraction and diffraction all occur. Diffraction patterns ordinarily overlap and obscure one another, and refraction data depend only on the distribution of indices of refraction in space -- complex and not particularly useful

information. It is reflection that is responsible for the visual perception of the sample.

To the degree that the crystals act as tiny mirrors, with surfaces inclined statistically at all possible angles, there will be specular contribution from the first surface of contact. The light from each initial interaction with the sample can either be reflected back to the detector or can encounter a new interface after travelling a short distance. Repeated encounters result in diffuse reflection, so the sample appears uniformly bright in all directions.

Note that the light reflected from the crystal surface has twice passed through the syrup film "filter." A doubling of the pathlength is inherent to the measurement; thus for scattering media, the Lambert-Beer law must be re-written with a factor of two: $I = I_0 e^{-2kx}$.

Sample particle size plays an important role in any diffuse reflectance measurement. Although in soft sugars the crystals themselves are far too large compared to the light wavelength for scattering to occur, the diameters of colloidal components in the syrup film are of the proper order of magnitude. Reflectance increases as the particle diameter is reduced to half the wavelength of the incident light, then drops off as the diameter approaches one-fourth the wavelength. Both cases can exist simultaneously in the syrup film. The fraction of the incident light scattered depends on the ratio of the absorption coefficient k , from the Lambert-Beer law, to the coefficient s from an analogous scattering expression. The two are related by the Kubelka-Munk function, which is beyond the scope of this article.

Some soft sugar properties that can affect the reflectance color are packing density, crystal form, absorbancy, and refractive index of non-sugars. Improper seeding, poor boiling technique, and small grain can contribute to diminished diffuse reflectance, as can raws high in polysaccharides, poor clarification, high calcium levels, and contamination by dextran or iron.

Hue

Any color that is not too saturated can be matched in appearance or specified quantitatively by a set of three arbitrarily chosen saturated colors called tristimulus values, reasonably well spread across the spectrum. This is why color TV systems use three kinds of colored dots* and color photography employs three emulsions. Indeed it is also why the retinas in our eyes contain three cone pigments. Note that the three colors do not have to

*"Black" seen on darkest TV scenes is simply the color of the tube face itself, really light gray.

be the "primary colors" -- red, yellow, and blue -- that children learn in school.

A reflectance system using, for example, green, amber, and blue filters could be used to specify exactly any soft sugar hue. However, reflectance measurement ordinarily uses a single filter and hence provides information about brightness and saturation only. As was noted above, a single measurement using, for example, a red filter indicates only a position on a red-green axis relative to shades of gray. For most purposes, this may suffice, so long as the hue is consistently reddish-brown. However, even subtle departures from the hue norm cannot be adequately characterized by such a system; it is possible for a product to be wholly within single-filter reflectance color specifications and at the same time display inferior color (Fig. 2).

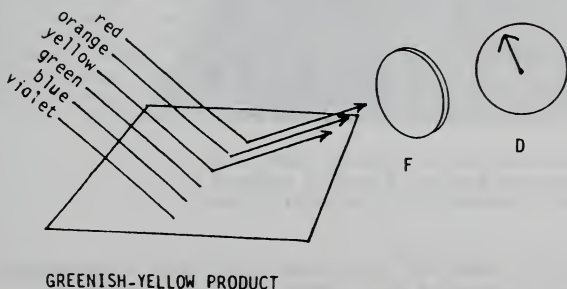
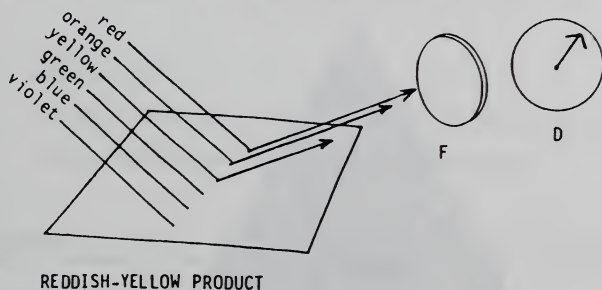


Fig. 2. Effect of subtle changes in hue on diffuse reflectance. F represents a 640 nm filter (red), and D the detector.

In order to specify our soft sugar hue for internal process control purposes, we use red, yellow, and green spectral absorbance colors**. The tristimulus system lends itself to triangular coordinate plots to specify a color position graphically. Since all sugars are overwhelmingly yellow, it is necessary to use weighted coordinates to mathematically enhance the red and green contributors, in order to obtain a useful result. This is done after normalizing the three absorbances to compensate for differences in saturation. The visual "red," actually red-orange, is computed using an empirical relation $(A_{480}-0.2900)*35$, with a default value of 0.3000 to avoid negative numbers. Visual "green," really blue-green, is computed as $(A_{675})*10$. Finally, the tristimulus values obtained are renormalized to obtain "hue-fractions" that can be plotted on triangular coordinates. Measurements are made without pH adjustment (Fig. 3).

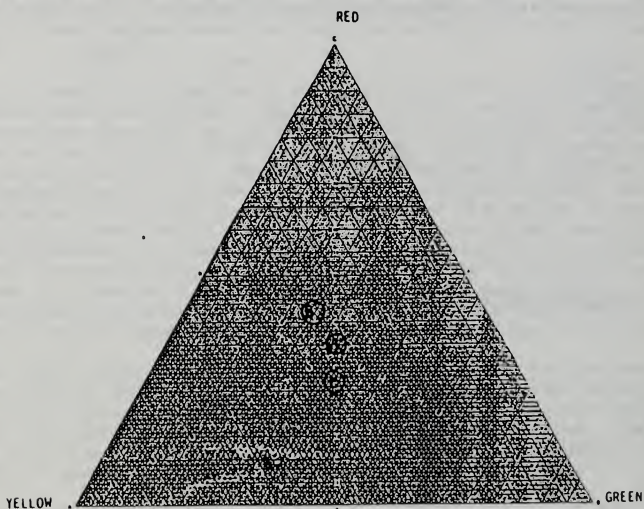


Fig. 3. Hue fractions of spectral absorbance colors. A=normal product, B=product reddened by autoxidation, C=product from low-activity bone char.

**"Spectral" colors refer to their characteristic wavelengths and not to any perceived hue. Thus light at 430 nm is indigo, whether or not any retinal cones or other detectors are in the vicinity.

It was noted earlier that scattering from colloidal substances in syrup film can affect diffuse reflectance. Such substances can also influence hue (Fig. 4). According to Rayleigh's law, the amount of light scattered varies inversely as the fourth power of the wavelength. Clear sugar solutions strongly absorb spectral blue-violet, but when a colorless scattering material, such as a polysaccharide, is added, the visual color is shifted slightly toward longer wavelengths:

visual color

white	blue	green	red	I
yellow	blue	green	red	II
redder	blue	green	red	III

- I. white light
- II. after passing through clear soft sugar solution
- III. after scattering by colloidal substances.

An analogous phenomenon is observed with sunsets, which are reddened by preferential scattering of blue light by atmospheric dust.

Illumination intensity can result in visual hue shifts. With increased illumination, colors become more yellow and blue, less green and red. Color saturation is also affected: as illumination intensity increases, saturation increases for all colors up to a maximum, then falls off with continued increase in intensity (Hoffman, 1958). These facts, largely due to the make-up of our retinas, were well known to Leonardo da Vinci, even though he never heard of rods and cones.

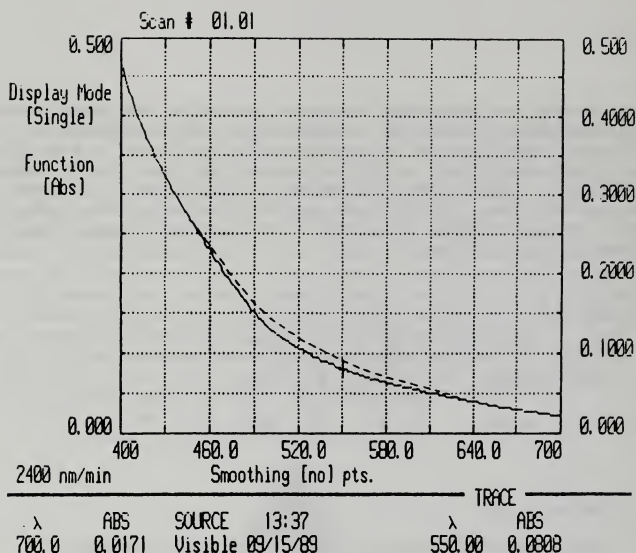


Fig. 4. Hypsochromic shift resulting from scatter. Solid line is spectrum of clear soft sugar solution. The dashed line was obtained with added corn starch.

Yellow and brown hues

Brown is not a color of the spectrum but originates at high absorbance levels of visible light. Imagine a perfect absorber, a blackbody, that reflects no incident radiation. If we gradually introduce imperfection that allows some reflection of orange light until color can be seen, the absorber appears not orange but brown. Reddish browns result from pinker oranges. Brown thus differs from orange in saturation and may be considered as orange mixed with a neutral color, such as gray or black.

The natural pigments of cane are yellow. Their spectra do not differ substantially from those of brown sugar liquors except in quantitative terms. Orangish tones probably result from quinone formation during processing. If a brown liquor is gradually diluted, a yellow-orange solution is obtained. Thus the brownness of sugar liquors results from orange reflection from highly absorbing media.

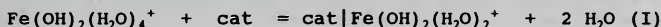
Greenness

In the case of greenish hues, generally considered undesirable, the presence of either magenta or orange plus blue-violet absorption are the principal sources, but the absence of cyan absorption can contribute as well.

The most important green colorants are believed to be ferric complexes of catechols such as chlorogenic acid (Riffer, 1986), which are ubiquitous in cane refineries although present at extremely minute concentrations. Despite its name, chlorogenic acid contains no chlorine atoms; the term, derived from Greek, does not mean "generator of chlorine" but rather "generator of greenness." In the absence of iron, chlorogenic acid in plant preparations forms a number of green substances by various reactions with, for example, amino acids or quinones.

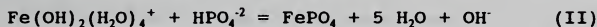
In the pH range 6-8, the solubility of ferric iron is about $10^{-8}M$, with the dominant species $Fe(OH)_2(H_2O)_4^+$. In sugar liquors, the solubility would be expected to be considerably higher because of the high affinity of trivalent iron for ligands that coordinate by oxygen, present both in sugars and non-sugars.

Although we have not determined the equilibrium constant for the iron-catechol complex, our studies indicate that the magnitude is $>10^{18}$.



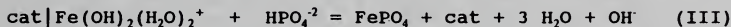
$$K_I = > 10^{18}$$

The deep color is associated with a bathochromic shift (toward the violet) accompanying increased covalent character. This can be reversed by adding phosphate, which is highly effective in decolorizing ferric complexes:



$$K_{II} = 6.7 \times 10^7$$

Since $K_I > K_{II}$, it might appear that phosphate would be an ineffectual decolorizer:



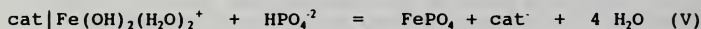
$$K_{III} = K_{II}/K_I = < 1$$

However, the acidity of catechol itself comes into play:



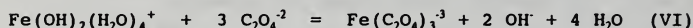
$$K_{IV} = 3.3 \times 10^{-10}$$

and the freed catechol is removed by anion formation:

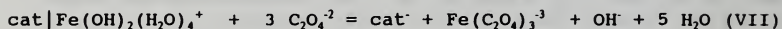


$$K_V = (K_{II}) (K_{IV}) / (K_I) (K_w) = < 2.21 \times 10^4$$

Formation of the oxalate complex is even more favorable:



$$K_{VI} = 10^{20}$$



$$K_{VII} = (K_{VI}) (K_{IV}) / (K_I) (K_w) = < 3.30 \times 10^6$$

Oxalate is a minor constituent of Hawaiian raws, present at levels of up to 45 ppm.

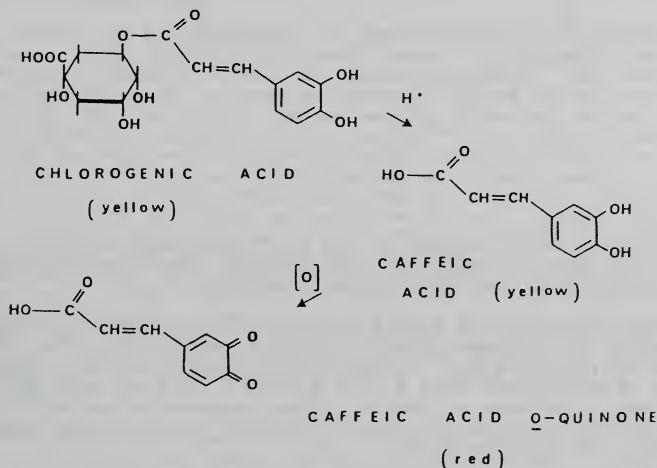
Greenness in a product can also result from low adsorbent activity; cyan absorbers appear to have a higher affinity for bone char than do their magenta counterparts. This phenomenon is discussed in the next section.

Origin of red colorant

The attractiveness of soft sugars results largely from reddish-brown hues absent in raws, which tend to be instead yellow-brown or golden brown. The importance of redness is demonstrated by the common use of red reflectance color to characterize soft sugars.

Redness in soft sugars might logically be attributed to the presence of substances which absorb blue and blue-green, or the absence of those which absorb red and yellow. Our tristimulus absorbance studies have shown the former alternative to be the correct one. Red reflectance measurement alone, however, cannot be used to distinguish between the presence of red and the absence of green, since these are seen as shades of gray in a continuum from black (green) to white (red). Therefore for our studies we used the tristimulus system described above.

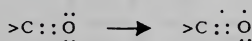
The red constituents were found to be generated in the vacuum pans and sweetwater evaporators. Although we have not isolated red colorant for characterization, a likely source is chlorogenic acid, present in all refinery streams and even in highest purity granulated. Under reduced pH and oxidizing conditions, such as are found in low purity vacuum pans and evaporators, this yellow compound is hydrolyzed to caffeic acid, also yellow, and appears to be subsequently oxidized to the red ortho-quinone:



The parent compound from catechol, likewise red, is unstable in aqueous systems.

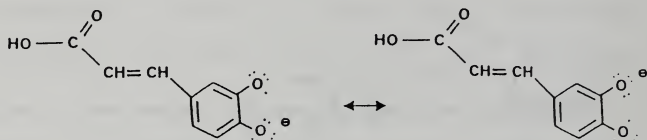
Quinones and catechols are also reportedly formed via base-catalyzed degradation of sucrose, a pathway unlikely in soft sugar components (Kato et al. 1973).

The high color of quinones compared to the acyclic version, 1,2-diketones, results from the carbonyls being locked into the same plane by the ring structure, no longer aromatic, which maximizes resonance. In both instances, an n to π transition occurs, involving excitation of a nonbonding electron from oxygen to a high-energy π orbital of the carbonyl system:



In the case of cinnamic acid derivatives, such as caffeic acid, the resonance path is further extended by conjugation.

The semi-quinone form, with the odd electron spread over the conjugated system,



would be unstable because of the non-equivalence of the two forms (Pauling, 1960). Thus the acid-to-anion interconvertibility of the parent compound is no longer possible.

The red colorants are readily adsorbed by bone char and might therefore be presumed to be of high pH sensitivity, that is, large indicator value. However, such sensitivity is a characteristic of substances with a high affinity for bone char but is not the reason for ease of removal, which appears to be due to the high diffusion rates of these relatively low molecular weight species and their polarity.

Quinones are particularly strong electrophiles and would be expected to be readily adsorbed at phosphate sites in bone char. The precursor phenolic functions are no longer present in quinones to exhibit indicator-type behavior with pH change. Quinones appear to be preferentially adsorbed when bone char activity is low, and this phenomenon can contribute to darker red reflectance and increased greenness.

Preferential adsorption of quinones on poor quality bone char could be explained by the fact that nearly all other colorants are nucleophilic, hence presumably adsorbed at calcium rather than phosphate sites. To carry this theory one step further, high calcium liquors would be expected to be somewhat redder off char, because inorganic polyvalent cations should diffuse to phosphate sites more readily than quinones, blocking those positions. We have not pursued this experimentally in the laboratory. However, the desirability of redness must be balanced against numerous problems associated with high calcium levels, most notably turbidity in certain liquid processed foods and the tendency to form scale on process surfaces.

During long storage, particularly under hot conditions, soft sugars gradually redden. We believe this is likewise a result of quinone formation, via various pathways including iron-catalyzed autoxidation. Quinones themselves have moderately high oxidation potentials, comparable to ferric iron, and probably participate in degradation via several routes.

Organoleptic properties

Aroma, flavor and mouthfeel are all important attributes of a soft sugar. What we ordinarily call flavor is not really taste but a blend of taste and smell or aroma sensations. The distinction can be confusing, because the aroma referred to is that experienced when the food is in our mouths. Chewing carries volatile chemicals, present at low levels, to the nasal cavity, where they impinge on 10^7 olfactory cells occupying just one square inch. The odor-sensitive tissues are blocked when a cold clogs our noses, so food seems tasteless but is really "flavorless." There are only four tastes -- sweet, sour, bitter and salty. Anything more complex we experience by virtue of our nasal receptors, not our taste buds. A fifth taste, usually omitted because it is unfamiliar to most people, is called by its Japanese name, "umami," and can be described as the taste of MSG, monosodium glutamate. Some substances have several tastes; for example, Epsom salt and sodium bicarbonate are each both salty and bitter. Some, like phenylthiourea, have a taste to only part of the population, which suggests that a genetic factor is responsible.

Consumers expect soft sugars to be consistent and to have full-bodied flavors free of harsh, bitter, or salty notes. Ideally the products would be made to some sort of flavor specification. This is not easily done, because sensory analysis is not only subjective but also susceptible to wide variations between people in sensory acuity. In addition, while instrumentation -- such as gas chromatography linked to mass spectrometry -- can detect and identify extremely minuscule traces of flavor components, such analysis tells us nothing about how the constituents are experienced by a human subject (Christianson et al., 1980).

This discussion will exclude turbinado sugar, which has sensory attributes very different from those of soft sugars. The differences are of two sorts -- the presence in turbinadoes of "green" flavors characteristic of not fully processed materials of plant origin (such as hydrocarbons, alcohols, and acyclic aldehydes) and the absence of important flavorants formed during refining (such as cyclic diketones and heterocycles -- thiazoles, oxazoles, and especially pyrazines).

Taste

Infants a few hours old respond favorably to sucrose but reject bitter, sour and salty tastes, which are potentially harmful. Thus a preference for sweet foods seems to be built into human physiology as a survival mechanism. Children have far more taste papillae than grownups, so foods undoubtedly taste different when we are very young, but we have no memory of this.

The different areas of the tongue seem to be relatively specialized. Sensitivity to sweetness is localized mainly at the tip, with salt-sensitive areas behind and sour-sensitive regions back further still. The bitter sensation is best detected on the back of the tongue.

The principal taste present in soft sugars is, of course, sweetness. Ash can contribute some slight saltiness, which is desirable when sufficiently subtle that the specific taste is not perceived. Organic acids present at low concentrations make up a sour component, which likewise must be at a low level to be pleasing. Bitter tastes, contributed by some phenolics, quinones, a few inorganic ions, and certain heterocycles, are clearly undesirable in a good soft sugar. What is desirable, apart from sweetness, falls under the category of aroma rather than taste.

Aroma

Although the sense of smell is not nearly so keen in humans as it is in lower animals, we can nonetheless discriminate between thousands of odors. Differences in acuity between people seem to be genetic; the inability to detect a particular class of odors has been shown to be inherited, suggesting the possibility of a deficient nasal receptor protein.

Although the four tastes function independently to some extent, it has yet to be demonstrated that different categories of smells are functionally distinct. Thus there is probably no special nasal receptor for "floral" aromas, for example. For the olfactory cells in the nasal cavity to be of thousands of different types would require a genetic complexity approaching that of the immune system -- and without the survival payback.

Unlike the names for taste qualities, those for odors are derived by association -- something smells like garlic, gardenias or cigar butts. Also unlike taste, smell figures prominently in association and memory: a slight odor can recall experiences which may have occurred many years earlier. Again unlike the sense of taste, that of smell shows little decline until about age sixty-five, although acuity can wane decades earlier in smokers. The decline observed in older people appears to hinge

more in impaired cognitive processing than it does on sensory disability.

Substances responsible for aroma must necessarily display at least some measure of volatility. It is obvious that such substances can be lost if a product such as soft sugar is overheated. Since flavorants are typically present at low concentrations, uniformity in aroma requires careful attention to processing conditions. Many flavorants are aldehydes, alcohols, acids, esters, and unsaturated compounds subject to reaction that can change their sensory properties in a fundamental way. The reactivity of these substances underscores the importance of moderate processing temperatures.

Mouthfeel

Mouthfeel properties are experienced independently from any specific taste or aroma. Such components include warming/cooling, smooth/powdery/gritty, astringent/alkaline, carbonated, metallic, and mouth-coating. Texture characteristics also include brittleness, dryness, and some special qualities associated with gels and foams. Pain receptors rather than taste buds may be involved, as with chiles. Senses other than tactile can play a role in cognition. For example, crunchiness -- of great interest to potato chip manufacturers -- has gustatory and auditory components as well as tactile ones.

Although sucrose cools upon dissolution, brown sugars -- as well as granulated sugars -- are ordinarily not eaten alone but in conjunction with other foods, so a specific cooling is not experienced. Moreover, a product of fairly high moisture content such as soft sugar would result in a minimal sensation of cooling for two reasons: the high heat capacity of water, and the fact that many sucrose molecules are already hydrated. The enthalpy of solution for sucrose, -5.52 kJ of heat evolved per mole, is small compared to the value, for example, of α -D-glucose, -10.7.

Viscous and fatty foods are responsible for mouth-coating and are of interest here only for high Brix brown sugar syrups. Grittiness is not perceived because of the very small crystals in our soft sugar conglomerates, and the syrup coating further softens the texture. Some refineries, however, do produce coarse grained brown sugars.

Metallic "tastes" in soft sugars are likely to be associated with iron contamination. One commonly speaks of such off-flavors as iron taste, although this is not strictly correct. There are no tongue receptors for metallic taste, nor do inorganics display an appreciable vapor pressure at ordinary temperatures that might contribute to a metallic "flavor." The sensation appears to be limited to transition metals and could result from an effect of d electrons on the surface potential of tongue receptors.

Raws typically contain well under 10 ppm of iron, present largely in entrained soil. Hawaiian soils are particularly rich in iron, as might be expected from their distinctive reddish color. However, these substances are very insoluble silicates, readily removed from the sugar during clarification or filtration.

Contamination that occurs during processing, from slow dissolution of iron surfaces, particularly in low pH streams, is in contrast very difficultly removed from soft sugars, because the iron forms very stable complexes with non-sugar components (Riffer, 1984). When iron levels reach 80 ppm, a metallic "taste" is frequently perceptible, although sometimes one merely notices an objectionable off-flavor that cannot be identified specifically as metallic.

Flavorants

In order to contribute to a flavor, component substances must display some volatility. If the vapor pressure of the material is zero at ordinary temperatures, there are no molecules to be carried to the nasal cavity. On the other hand, the thresholds for detection of certain flavorants can be exceedingly low, so relatively few molecules must impinge on olfactory receptors to produce a sensory response. Differences in threshold perception enormously complicate sensory profiles, because minor constituents can be of greater significance than major ones. Hence a complete chromatographic separation, with qualitative and quantitative analysis of all of the volatiles in soft sugar sample, does not suffice to characterize a flavor. One technique used to normalize flavorant contributors is to express them in odor units rather than in concentrations:

$$\text{odor unit} = \frac{\text{concentration of component}}{\text{threshold of that component}}$$

Such characterization, of course, requires sensory testing to determine perception thresholds.

The chemistry of caramelization, the source of many sugar flavorants, has been described in detail elsewhere (Greenshields et. al, 1972) (Hodge, 1965). Under acid conditions, present in low purity processing, the production of furfural derivatives is favored. Numerous volatile products are formed.

The Maillard reaction between amino acids and reducing sugars, to yield volatile pyrazines, is a rich source of flavorants in cooked or browned foods. However, in soft sugars the very low levels of proteinaceous substances present limit the importance of this pathway. Although even refined sugars can contain

measurable levels of protein, most such material is removed during phosphatation. Despite the low concentration of pyrazines, they nonetheless appear to have a strong influence on flavor, as we will see below.

The important flavorants in soft sugars are formed largely during refinery processing and are for the most part not present in raws, which is why turbinado sugars display sensory properties very different from those of soft sugars. For this reason, refining know-how and raws of consistent quality are essential to the manufacture of high quality soft sugars. Refinery pathways to flavorants include Strecker, thermal, and microbiological degradation.

Godshall and Roberts had an expert taste panel evaluate 27 different soft and turbinado sugars (Godshall et. al, 1980) (Godshall, 1981) (SPRI Technical Report No. 13, 1985). The identifiable characteristics of highest intensity were char, molasses, and licorice -- not necessarily positive attributes. Desirable flavor notes, such as caramel and butterscotch, were judged to be too low to contribute to the flavor score. "Green" flavors could be discerned only in the turbinado samples, as one would expect.

They found that flavor scores correlated best with amino nitrogen, presumably from pyrazines. Except for such Maillard products, most of the flavorants are colorless molecules. Hence the association between dark color and rich flavor is at least to some degree psychological, although there is to be sure a correlation between color and non-sugar content. This association is distorted for those refineries that use added caramel color in their dark brown sugars, since this additive has low volatility and can contribute harsh flavor notes.

Godshall found acetic acid to be the most abundant volatile constituent in all light brown sugars studied (SPRI Technical Report No. 9, 1983). Concentrations in all samples analyzed were well above the taste and odor thresholds. Acetic acid originates in low purity sweetwater, from bacterial activity. She found that dimethyl sulfide, responsible for molasses flavor, originates in the cane plant. On the other hand, caramel flavorants, such as maltol and acetyl formoin, as well as butterscotch contributors, such as diacetyl and 2,3-pentanedione, result from sucrose degradation reactions.

Although we are not aware of soft sugars being manufactured using ion exchange resins, such processing could introduce traces of amines that might impart fishy flavors to the product. However, these could very likely be removed by a subsequent treatment with activated carbon, at levels sufficiently low that desirable flavorants are affected only minimally.

Beet factories manufacturing soft sugars must incorporate cane molasses into their products, because the corresponding beet product is notably unpalatable.

Sensory Testing

A "taste" panel is more accurately described as one for sensory testing, since ordinarily one is not interested explicitly in one of the four tastes, particularly when a complex flavor such as that of a soft sugar is to be evaluated. We at C&H have a permanent pool of about 20 employees, volunteers from all levels in the company, who have been screened for acuity of sensory perception and given specific sensory training. It has been demonstrated elsewhere that individuals vary greatly with regard to the minimum concentration that is barely detectable. And it is also known that women have a keener sense of smell than do men.

The testing we found most suitable for our needs is discriminatory testing, in which panelists are asked which of three samples is different from the other two. More sophisticated differentiation, such as being able to assign a numerical score to a flavor, requires a very intensive and lengthy training program, with frequent retraining "booster shots" to keep panelists' abilities from waning. We were unable to justify this, because it would require employees to be absent from their regular jobs frequently and for extended, indefinite periods.

Neither do we ask panelists to articulate any perceived difference in their own words, since the vocabulary used would be very difficult to standardize without intensive training. Instead, they are asked to estimate the degree of difference between the duplicate samples and the odd sample, and whether specific flavors can be detected: caramel, charred/burnt, molasses, butterscotch, licorice, sour, and metallic. The evaluation sheet is patterned after a prototype developed by M. A. Godshall (SPRI Technical Report No. 13, 1985).

Panelists are also asked to express a preference for either the duplicate samples or the odd sample. All of these data are obviously applicable only for those individuals correctly identifying the odd sample. So called "hedonic" testing -- like, dislike -- is not particularly useful for the kinds of subtle distinctions we are seeking to make.

When given samples to evaluate, panelists go into test booths isolated from outside sensory stimuli. The booths are illuminated with red light, so that the subjects are unable to use sample color as a clue. Samples are ordinarily presented in dilute solution, typically 15° Bx or lower.

We have found it difficult to get panelists to disregard sweetness, which is, of course, the strongest sensory property of soft sugars, but one that is not very interesting from the perspective of soft sugar profiling. The sugar matrix can also mask or modify other flavorants.

PHYSICAL PROPERTIES

We monitor our soft process by the usual analyses of purity, Brix, and color. Color data and moisture content have been found to be more useful for control purposes when expressed as units per unit non-sugar, since it is the non-sugars that are of most significance in soft sugar quality. We also monitor the ratio of invert content to specific conductance. Such control helps assure good product texture, free of stickiness.

Crystal size

The advantage of using conglomerates of fine crystals in order to be able to incorporate relatively high syrup levels into the product is obvious from the familiar relation between particle size and surface area. The syrup coating is, of course, rich in color and flavor and is what distinguishes soft sugars from granulated ones. P. G. Roza of our staff was able to demonstrate a positive correlation between poor crystal size uniformity and occurrence of syrup balls. This appears to be related to purging characteristics: poor uniformity would result in impeded syrup flow in the centrifugals. In addition, a relatively high level of large crystals would diminish the surface area available for syrup adsorption.

Syrup ball incidence also shows a correlation to the ratio of invert content to specific conductance in soft liquor. An optimal ratio is observed, which suggests that more than one principle is at work here. A large spread in crystal size distribution results from, among other things, too high a level of supersaturation in the vacuum pans.

Moisture content has an important bearing on keeping qualities. If the level is too high, moisture will be released to the atmosphere; and this evaporation will result in crystallization in the syrup film, producing caking. Too low a moisture level is a consequence of sugar purities that are too high. For process control, in our experience it is more useful to monitor the ratio of moisture to non-sugar solids than the absolute moisture content.

Typical analyses of C & H soft sugars

<u>Component</u>	<u>Light Brown</u> (Golden C)	<u>Dark Brown</u> (Yellow D)
Sucrose	89.2%	88.4%
Moisture	3.4	3.2
Invert	3.4	3.7
Ash	1.6	1.7
Organic non-sugars (by difference)	2.4	3.0

MICROBIOLOGICAL PROPERTIES

Processing temperatures suffice to pasteurize soft liquors, destroying yeast and mold. A polish filtration reduces bacterial concentrations to insignificant levels. However, ordinary processing obviously cannot yield a sterile product.

Nutrients needed for microbiological growth, such as phosphate for DNA replication, are in plentiful supply in soft sugars, so the factor limiting growth is osmotic pressure. It is possible to produce soft sugars with a moisture content sufficiently high to allow some yeast spoilage. Since a surplus of sucrose is always present, the resultant osmotic pressure will depend on the relationships between ash, invert, and moisture contents. Molds are genetically programmed for very high moisture conditions and are unlikely to pose a problem unless product accidentally becomes wet.

Any dextran formed during processing is likely to be concentrated in soft liquor, so careful attention must be paid to sanitation. Residence times in process, particularly of low temperature, low density streams, must be minimized. Polysaccharides, either indigenous to cane or of bacterial origin, can adversely affect product appearance and tend to darken reflectance color, for reasons described above.

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DISCUSSION

Comment: We have two refineries; both produce soft sugar, and each is a little different. When customers require certain product characteristics, and we try to define what we are measuring, we find these correlations between property and measured value difficult to rationalize. You've given us a lot to think about in this regard.

Question: My company makes soft sugars by a vacuum process. We measure two properties that you have not mentioned. We measure apparent density. Can you also differentiate the volume of soft sugar in packaging from the apparent density measurement.

Another property we measure is temperature before packaging, because water migration in the package depends on temperature, and it's important to stop this and inhibit caking within the package.

Riffer: These parameters are of course of interest in our process too. What I'm talking about today are really the characteristics that are used in product specification, rather than in process control.

Question: What is your opinion about the effect of ion exchange resins on soft sugar flavors? When we put a new batch of ion exchange resin into process, we avoid making soft sugars for one week, but after that time find no problems.

Riffer: That's very interesting. I wasn't aware of such measures.

Question: Congratulations on an outstanding and timely paper. I have two questions. First, about your test panel to taste products: can the panel give you any indication of the process changes you will need to make prior to, the production of a product?

Riffer: I think you are suggesting that we have samples of sugars that are not our regular production; no, we test only the normal production runs.

Question: Secondly, the most important aspect of soft sugar sales is, to many minds, flavor. Has any work been done by your group to determine if any aftertaste is triggered by any component of brown sugar flavor.

Riffer: We have not looked specifically at aftertaste.

Question: My question deals with handling characteristics. By your analysis, there is about 7.5% non-sugars in your soft sugars. Do you feel that there is an upper limit to the amount of non-sucrose solids you can put in a soft sugar?

Riffer: Yes, I'm sure there is, but I can't give you an exact figure.

Question: Is there any recommended reading material for those of us just starting in sensory testing?

Riffer: There are some semi-popular articles that are easily understood.

SIMULATED MOVING BED TECHNOLOGY APPLIED TO THE CHROMATOGRAPHIC RECOVERY OF SUCROSE FROM SUCROSE SYRUPS

Mike Kearney

The Amalgamated Sugar Company

Unlike the familiar batch chromatography column, the simulated moving bed system is designed with an internal circular flow. No configuration requires the development of an equilibrated separation waveform within the column. The waveform can be adjusted with respect to a large number of parameters. Although description and modeling of a simulated moving bed can be difficult, the circular structure results in several unusual and beneficial operating characteristics when applied to sucrose syrups.

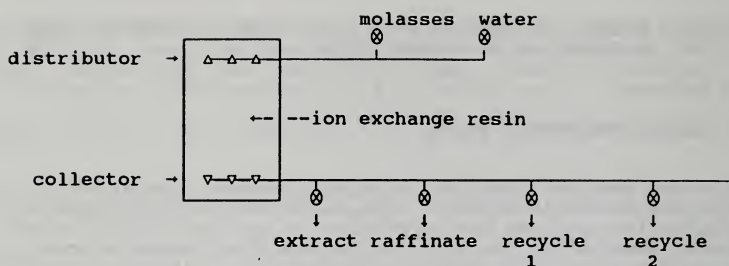
Bench vs. Industrial Scale Chromatography

The major goal of a sugar factory is to separate sucrose from nonsucrose components. Methods include diffusion, liming and carbonation, crystallization, Steffens process, etc. On a bench scale, sucrose can be separated from nonsucrose solutions using standard liquid chromatographic techniques. Are any major changes in basic concepts necessary to scale-up chromatography for sugar factory applications?

The goals are certainly different for the scaled-up process. The bench scale is usually concerned with identification of components. The industrial process will be concerned with obtaining large quantities of separated products. The bench system may require good baseline separation. This will not necessarily be important to the sugar factory. Such considerations have led to special operating procedures for simple scaled-up bench systems (batch systems). Operating configurations far removed from bench scale concepts have also been developed.

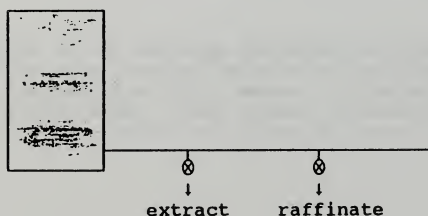
Batch Systems

The batch is the simplest scaled-up type and is the easiest to understand. The operating principles are very similar to the bench operation. Typically a column will be filled with an ion exchange resin in monovalent cation form. The column will contain a top distributor and bottom collector.

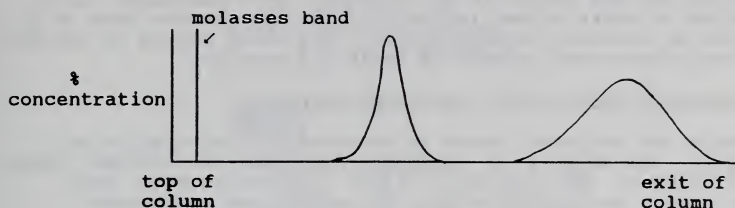


The diameter of the column may be a few meters, so distributor design is important. Plug flow through the resin bed must be maintained to avoid disturbing the development of the separation profile. Operating steps are conceptually similar to bench scale: (1) Feed a narrow band of molasses to the column, (2) Elute the molasses with water, and (3) Collect products in sequence as they exit the column.

A second band of molasses can be added to the column when enough molasses free resin becomes available at the top. In the figure below, the column is illustrated as three separate feed bands being separated simultaneously.



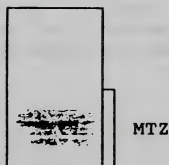
Illustrated another way:



Sucrose is preferentially absorbed by the ion exchange resin and therefore separates by lagging behind the majority of the non-sucrose components. The first nonsucrose portion of a given profile is removed through a valve and designated "raffinate." The tailing sucrose portion of the profile is then removed as "extract." In reality, recycle cuts are also taken and returned, for example, to dilute molasses. The reason for this is that baseline separation is not economically justified. By treating large quantities of feed material and cutting out the useable portion of the separation profile the process becomes viable on an industrial scale.

It is noted that a more complex set of valving would allow for removal of additional product cuts of the separation profile. One example is the removal of betaine which tends to lag behind the rest of the profile.

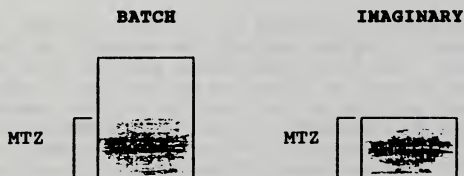
In a batch system a large volume of water phase resin must be available at the top of the column before feeding in a second band of molasses. While in the water phase, this resin serves no useful purpose with respect to the separation. Only those areas where molasses solution is present are doing any useful work. The area where separation is occurring is referred to as the mass transfer zone (MTZ).



The non-working volumes are, to a certain extent, large water and resin storage areas. This is not an efficient use of resin, although the MTZ length in an industrial chromatography column relative to total column length is certainly greater than on an analytical oriented chromatography. The bench system is extremely inefficient with respect to resin utilization.

An Imaginary Improvement over Batch Operation

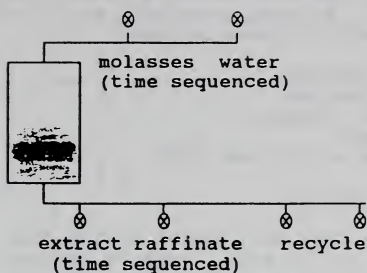
Although the scaled-up batch is successful, a more efficient operation can at least be imagined. Consider two columns, batch and imaginary. The following figure illustrates both, each containing a separation profile of sucrose and nonsucrose.



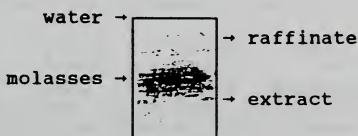
The imaginary system has no wasted resin, i.e., the mass transfer zone covers the entire column. Therefore less resin and equipment is in use.

In addition, suppose the imaginary system does not require feeding a band of molasses at the top and then eluting down the column. Instead, assume that the separation profile always exists at a steady state. Feed and water are continuously added to the column and product extract and raffinate are continuously removed. The following figures compare the batch and imaginary continuous system:

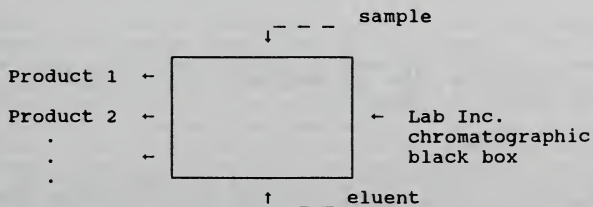
BATCH SYSTEM



IMAGINARY CONTINUOUS STEADY STATE SYSTEM



It may be interesting to imagine using such a chromatographic device in the laboratory. Rather than injecting a microliter sample and eluting, sample and eluent would enter continuously with products exiting continuously.



How can such a device be built?

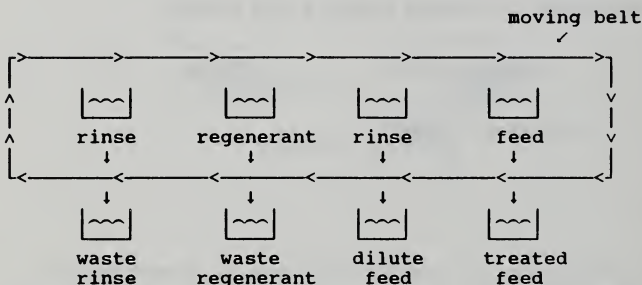
Counter-Current Concepts

The starting point for such a device is the concept of counter-current operation. Several industrial scale counter-current schemes are available. These include:

1. Moving equipment
2. Pulsing systems
3. Carrousel systems
4. Moving bed
5. Simulated moving bed (SMB)

1. Moving Equipment

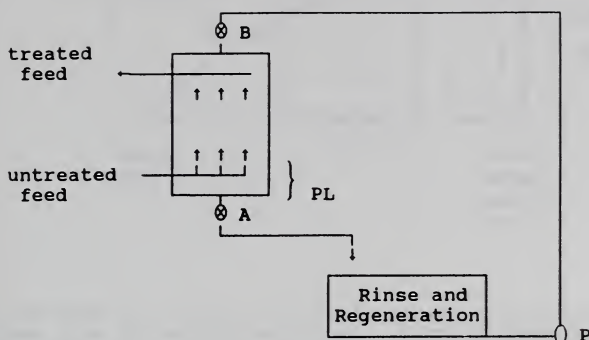
An example of this type is a porous moving belt with active material adhering to the belt. Feed is treated by the active material on the belt. The belt is then rinsed and reactivated.



The system is continuous with the equipment moving counter-current to the inputs and outputs.

2. Pulsing Systems

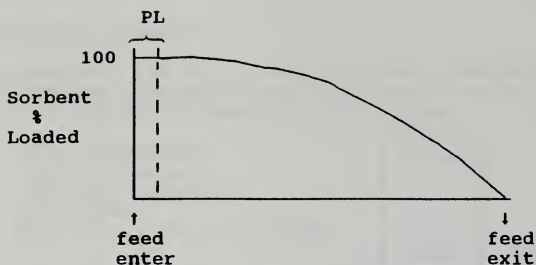
The following figure illustrated a pulsing system for which sorbent bed material moves counter-current to the treated material. This could represent, for example, corn syrup treated through a carbon column.



Untreated feed enters the bottom, flows up through the sorbent and exits out the top. When a certain level of undesired material (perhaps color) begins to leak from the top collector, the following actions take place:

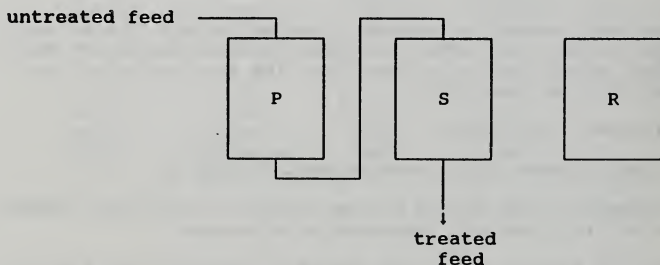
- Valves A and B open
- Pump P pumps reactivated sorbent through B
- Sorbent in the bottom of the column is displaced through A to the rinse and regeneration operation.

The amount of sorbent displaced from the column at one time is measured by the pulse length (PL in the figure). The pulsing occurs every time the leakage of observed component reaches a threshold level in the treated feed. Such a system is much more efficient than removing the entire column of sorbent for regeneration when the threshold leakage occurs. Instead only a small fraction of the total bed is regenerated, and most importantly, this small fraction represents the most exhausted portion of the bed. The less exhausted sorbent at the top of the bed incrementally pulses down the column eventually becoming totally exhausted, and only then regenerated. The system could be made even more efficient by operating the rinse and regeneration modes in a pulse manner. The following figure illustrates the pulsing process:



3. Carrousel Systems

A similar counter-current design is the carrousel or "merry-go-round." This is very useful in ion exchange applications. The following is a three column ion exchange system operating as a carrousel:



P = Primary column

S = Secondary column

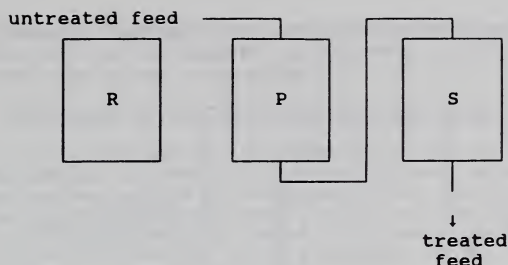
R = Regeneration and standby column

When leakage appears in the treated feed, the following takes place:

S becomes P

R becomes S

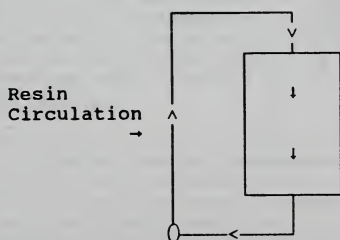
P is regenerated and placed in stand-by (P becomes R)



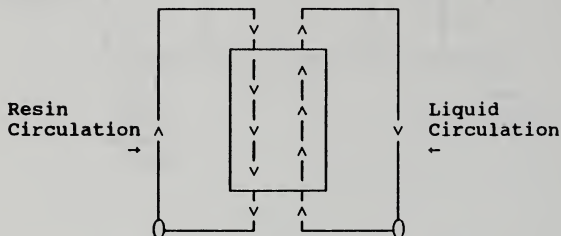
Note that only the highly exhausted primary column is regenerated. This type of configuration will result in more efficient use of regenerant and smaller installation requirements. The concept simulates counter-current movement of the sorbent beds. The operation becomes more efficient and simulates continuity to a greater extent as more and more columns are added. However, the improvement is smaller and smaller with the addition of each column.

4. Moving Bed Chromatography

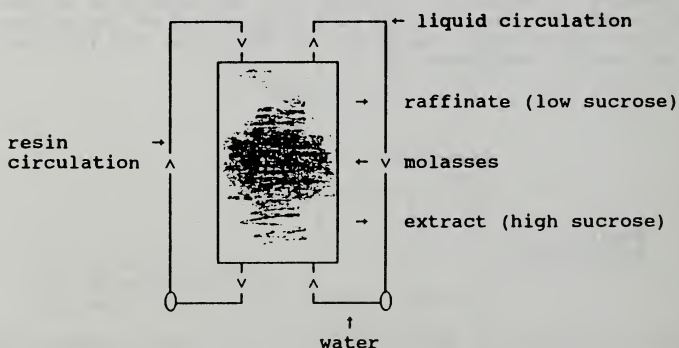
Having outlined the benefits of a few types of counter-current configurations, the focus will now be on a design applicable to chromatography of molasses. A column will be completely filled with ion exchange resin. A pump will be provided at the bottom of the column which pumps the resin out the bottom and into the top of the column. The pump is continuous.



A second pump is provided to pump liquid counter-current to the resin:



Molasses is fed continuously to the center of the column. Because sucrose is preferentially absorbed by the resin, it tends to move down the column. On the other hand, nonsucrose tends to move with the liquid flow up the column. Collectors and valves can be placed near the ends of the column to continuously collect the high sucrose extract and the high nonsucrose raffinate. Water must also be added continuously at the end of the column:

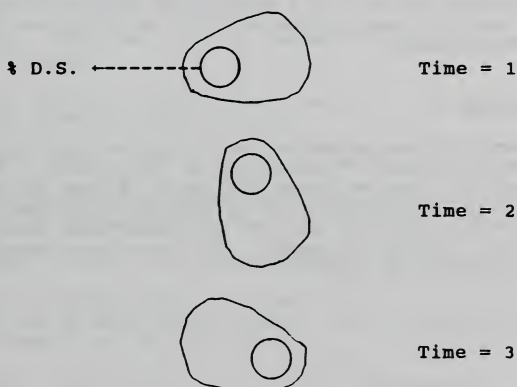


Note that this chromatographic system provides the results of the imaginary chromatograph discussed in an earlier section. These benefits include continuous operation and an MTZ which is the length of the column.

In addition only the tail end portions of the steady state separation profile are removed as products, so no recycle of poorly separated syrup is required.

5. Simulated Moving Bed Chromatography (SMB)

Because of the difficulties involved with pumping resin, the moving bed design is usually simulated in some manner. The resulting systems act as a moving bed. The same steady state separation profile develops with the use of the SMB, however the profile will usually rotate around the system path. This is illustrated as follows for three snapshots of the separation profile, each at a different time:



In addition to the advantages of moving bed (or SMB) outlined in the last section, there are several other unusual and beneficial characteristics in comparison with batch. Two examples are control of inventory and control of traveled path length.

Inventory Control

In a batch system a given quantity of feed material enters the top of the system, passes down and out the bottom. If all the feed and water were mathematically composited during a day of operation, it would be found that the composited % DS and % purity entering equals the composited % DS and % purity exiting. This is a simple material balance for which $\text{in} = \text{out}$. In addition if all material within the column were composited over a period of time this composite % DS and % purity also = $\text{in} = \text{out}$. So for a batch system:

% DS and % Purity (all entering material composited)	=	% DS and % Purity (all internal material composited)	=	% DS and % Purity (all exiting material composited)
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For a moving bed (or SMB) chromatograph as described earlier this is not true. At steady state in = out by material balance but in = out \neq internal. This is because the moving bed system creates an inventory of material as it approaches steady state (this also means in \neq out as steady state is approached). The important result is that for a given feed and water rate and composition, there are an infinite number of possible steady state separation profiles (or inventories) within a moving bed system. This leads to a variety of useful steady state extract and raffinate % DS and % purity combinations.

Path Length Traveled

In a batch system entering molasses has no choice but to pass through a bed length equal to the height of the column. If a column is 40 feet high the molasses will pass through 40 feet of resin before exiting. This is not true with a moving bed. Path length traveled before exiting will be dependent upon the flow rate of circulating liquid and resin. This provides opportunity for enhancement of product quality.



Speed of profile rotation determines
path length traveled

CONCLUSION

Scaled-up bench chromatography for use in the sugar industry has encouraged the development of continuous-counter current simulated moving bed configurations. Successful designs are now in use for desugarization of molasses.

DISCUSSION

Question: When you separate molasses with a purity of, say, 60, what is your recovery of sucrose in the product fraction and in the non-sugar fraction? What purities are these two products?

Kearney: That's a very important question. The two materials coming out of the separator also have to go to the sugar end to be recovered. It's important to specify "recovery across the separator" and actual product after it's gone through the sugar end again: it's very typical to see a sugar fraction of 93 purity, across the separator, from 60 purity molasses. There should be 90% or more recovery of sucrose across the separator also. That then passes to the sugar end. On one pass through, there should be an 80% recovery of crystallized product. That's only on first pass through.

Question: How much resin is needed for production of one ton sugar per hour?

Kearney: Typical systems run from 160 - 250 kg of non sugars, per cubic foot of resin per day.

Question: What is the process operating temperature?

Kearney: Typically around 80°C. That's necessary to prevent any microbiological problems, while still being safe for the resin.

Question: Do you have process plants in operation or only pilot?

Kearney: Process scale. We built one in Japan four years ago. We have two in Twin Falls, Idaho. We are processing almost 350 tons molasses per day in Idaho.

Question: Can one visit to see this equipment?

Kearney: Yes.

Comment: We installed an ion-exclusion plant last year, with the simulated moving-bed system. With regard to the previous question about amount of resin: about 200 cubic meters of resin are used for 65 tons extra sugar recovered per day. We are very satisfied with the plant - it works very well indeed. Our greatest problems - after understanding what a simulated moving-bed was - was trying to explain the principle to other people. Mr. Kearney today has made a very clear explanation.

Question: Is the moving-bed technology also applicable to cane refinery molasses, or cane raw molasses, where we have to separate several fractions, including the invert fraction?

Kearney: Yes - that's an interesting and different problem. The molasses composition is different; the purity is quite low. There is an economic consideration based on the amount of sugar that can be recovered. Cane molasses is a more difficult feed than beet molasses, because of its physical properties, but once the preparation problem is overcome, it can be separated by this process.

PREPARATION OF MICROBIAL POLYFRUCTOSE (β -(2-6) FRUCTAN) FROM SUGARBEET AND SUGARCANE JUICES AND MOLLASSES

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Sugar Processing Research, Inc.

INTRODUCTION

As part of an ongoing study to develop new products from the agricultural resources provided by sugar-producing crops, a search was initiated for microorganisms to produce polymeric compounds for industrial use.

Polysaccharides were the first group of polymers considered. Dextrans, polymers of glucose synthesized from sucrose, are important industrial polysaccharides (Meade & Chen, 1985).

Fructans are natural polymers of fructose. Depending on the linkage types, fructans are classified into two groups: the levans, with mostly β -(2-6) linkages and the inulins with β -(2-1) linkages, as shown in Figure 1. Many fructans of both types have branched chains. Levans and inulins of low molecular weight are abundantly found in plants, while high molecular weight fructans are produced by many microorganisms (Avigad, 1968; Pontis and Del Campilo, 1985; Vandamme and Derycke, 1983).

A variety of microorganisms produce extracellular polysaccharides in the form of capsules attached to the cell wall, or as slime secreted into the growth medium. These materials are used in the organism's defense mechanism, or as a food reservoir. Some bacteria produce fructan, among which Bacillus spp. predominate. Oral bacteria such as Rothia dentocariosa, Streptococcus salivarius and Odontomyces viscosus accumulate fructan in human dental plaque (Higuchi, et al, 1970; Manly and Richardson, 1968; Newbrun, 1969). Several species of yeast and fungi are also known to produce levan (Fuchs, et al, 1985; Loewenborg and Reese, 1957). Most research on the biosynthesis of fructan has been conducted using Bacillus subtilis, Aerobacter levanicum, and Streptococcus salivarius (Dedonder, 1966; Tanaka et al, 1981; Hestrin et al, 1943; Mantsala and Puntala, 1982; Perlot and Manson, 1984; Yamamota et al, 1985; Fuchs et al, 1956; Lyness and Doelle, 1983; Evans and Hibbert, 1946; Feingold and Gahatia, 1957; Takeshita, 1973). Recently, fructans produced by Zymomonas mobilis (Barrow et al, 1984; Mays and Dally, 1985; Kennedy et al, 1989), have been investigated.

Microbial fructans (or levans), like dextran, were first found in sugar factories (Fuchs, 1959; Avigad, 1968; Schneider et al, 1969). These polysaccharides caused difficulties in the beet sugar manufacturing process by increasing the viscosity of

fructans have received little attention and have never been exploited for industrial uses.

ISOLATION OF A FRUCTAN-PRODUCING BACTERIUM

Figure 2 shows the production scheme using a levan producing bacterium. About 1 g of rotting sugarcane stalks and the adhering soil particles were added to 100 ml of basal medium and incubated at 30°C with constant shaking. The isolation medium consisted of sucrose 80g to 150g; peptone, 2g; yeast extract 2g; K_2HPO_4 , 2g; $(NH_4)_2SO_4$, 0.3g; in a liter of water. The growth culture was then transferred to fresh media every 7-10 days. A detailed isolation procedure was reported elsewhere (Han and Clarke, 1990) and the organisms have been registered at USDA, Northern Regional Research Center, Peoria, Illinois, and identified as NRRL B-18475 and B-18476. These, plus several other strains, are discussed in this paper.

PRODUCTION OF POLYFRUCTOSE

The *B. polymyxa* (NRRL B-18475) produced a large quantity of fructan when grown on 4%-16% sucrose solution. The organism converted the fructose moiety of sucrose to fructan; of the remaining glucoses, most were used as the carbon source for microbial growth and a small amount accumulated in the growth medium. No fructan was produced when the organism was grown on glucose or fructose.

The composition of the products was monitored by HPLC (Sugar Analyzer, Waters Associates; HPX-87C column, BioRad Corp. with deionized water, 40 ppm as mobile phase). During fermentation, the sucrose levels dropped and fructan started to appear in 2 days; thereafter the, sucrose level gradually decreased as fructan increased. Glucose was the major byproduct. The pH of the growth medium fell from 7.0 to 4.7 indicating acid production. In reports of other fructan production, maintaining pH above 5.5 was important because the optimum pH for fructansucrase is between 5.5-7.0 and fructan may be hydrolyzed at a lower pH (Avigad, 1968). Optimum temperature for growth and fructan production was around 30°C.

Composition of a typical fermentation mixture is shown in Figure 3, and progress of fructan formation over an 8 day period is shown in Figure 4. Optimum period for production of high molecular weight fructan appears to be 3 days. Fermentation times were extended to 16 days, with little improvement in yield. The detailed production is reported elsewhere (Han and Clarke, 1989). Conditions of ionic strength and pH are under investigation.

Fructans can be produced from sugar juices (sugarbeet juice and sugarcane juice) and molasses; typical yields are shown in Table 1. These are production yields on juice and molasses added instead of sucrose to the growth medium. Beet and cane molasses were not treated or cleaned-up (e.g. desludged) in any way in this set of trials, therefore the crude product reported here from molasses was yellowish in color, and required a clean-up procedure, such as filtration over DEAE-cellulose, to produce a white product with a slightly lower yield.

STRUCTURE AND PROPERTIES

Experimental methods and materials used in determining the structure of this fructan, or levan, which has been given the trivial name of polyfructose, are reported in detail elsewhere (Clarke et al, 1990). Carbon-13 nmr spectra (as shown in Figure 5) indicate that all fructose molecules were in the same conformation (Clarke et al, 1990). Proton nmr have indicated that only fructose is present in the molecule. Because sucrose is the initial molecule in the chain, there must be some terminal glucose residues. However, since the molecular weight of polyfructose has been shown to be about 2×10^6 daltons, the ratio of fructose to glucose is about 12,000:1, and so glucose present would not be observed on nmr spectra or by HPLC analysis. Comparison with literature assignments of nmr peaks indicated that polyfructose is of the β -(2-6) linked type (Barrow et al, 1984). Methylation analysis with GLC and MS detection indicated that ca 71% of the fructose moieties form a β -(2-6) linked backbone, with 12% branch points of β -(2-1) linkage, and 13% terminal groups (Clarke et al., 1990; Lindberg et al., 1973).

Polyfructose is not hygroscopic; and lyophilized material has been stored under ambient conditions (25°-30°C; 70-90% relative humidity) for several months. It is soluble in water, a low concentration although some turbidity or opalescence is always present. This opalescence is apparently characteristic of fructans in solution. Addition of a few drops of acid has been observed to remove the opalescence while not causing any hydrolysis or breakdown of the fructan. It can readily be hydrolyzed in acid below pH 3.5 to form fructose. The addition of heat (standard or microwave) greatly increases the rate of hydrolysis, as shown in Table 2.

ENZYME HYDROLYSIS

It was of interest to conduct enzyme hydrolysis of polyfructose to gain more information on the structure. The polysaccharide showed no reaction with amylase, dextranase or other glucanases (exception below).

No fructofuranosyl fructosidase, or fructanase, could be obtained, so various available crude enzyme preparations were presented to the polysaccharide. Two of these commercially available enzymes, Gamanase (Novo Biochemicals) a hemicellulase, and Promozyme (Novo Biochemicals) a "debranching enzyme", or crude pullulanase, had similar effects on polyfructose. Both hydrolyzed the polymer to a smaller polysaccharide of about 20,000 daltons. Gel permeation chromatograms for molecular weight determination (Sephacryl S-500; water) are shown in Figure 6. It should be noted that HPLC (Clarke et al, 1990) chromatograms of the fermentation mixture showed two peaks (or a split peak) for fructan; similar HPLC analysis of the smaller fructan (now referred to as low molecular weight fructan or levan) showed a single peak. Apparently a fructanase exists as a contaminant in the commercial enzyme preparations used, and is similar to a fructanase present in the strains NRRL-B-18495 and NRRL-B-18476. Carbon-13 nmr spectra of the enzyme hydrolyzed products, as shown in Figure 7, were similar. Comparison of nmr spectra (200 MHz; TMS external standard) as shown in Figure 8 indicates, by the increase in terminal groups that the enzymes have hydrolyzed 8-(2→6) linkages in the polyfructose backbone, to form smaller backbone segments that maintain their branch structures.

The lower molecular weight levan is much more soluble than the high molecular weight, giving a clear solution. Differences in rate of acid hydrolysis of the high and low molecular weight products are shown in Table 3, and differences in viscosity of solutions of the two in Figure 9. Angular rotations and melting points are listed in Table 4.

APPLICATIONS FOR POLYFRUCTOSE

Many applications for polyfructose in the food and beverage industry are possible.

Polyfructose synthesized by the isolated strains of B. polymyxa provides a low cost source of fructose, in a stable form that can be stored, made from sucrose. The non-hygroscopic nature of the high molecular weight polymer is an unusual property for a fructose compound. Hydrolyzed solutions of polyfructose are, in effect, fructose syrups. The question of the use of this compound as a liquid sweetener source is an economic one. Polyfructose in its lower molecular weight form offers the possibility of sweetness potentiation.

The viscosity profiles of polyfructose indicate that it can be used as a thickener, perhaps combining added sweetness and enhanced mouthfeel in a single product. Some applications and properties are outlined in Table 5.

Polyfructose can be used to encapsulate flavoring and coloring agents. As one form of encapsulation, it can be made into tablets containing flavor, color or other additives which will store well under atmospheric conditions because polyfructose does not absorb water readily. Polyfructose is a stable, non-toxic, non-hygroscopic compound that is expected to be digested in the stomach, contributing calories equivalent to its hydrolysis product, fructose.

SUMMARY

A microbial polysaccharide that is a polymer of fructose has been produced from sucrose in good yield and high purity by a strain of Bacillus polymyxa. The compound, which has been given the trivial name polyfructose, consists entirely of fructose with one glucose unit in the initial sucrose terminal group.

The structure has been shown by nmr and methylation analysis to be a β -(2 \rightarrow 6) linked backbone with up to 12% branching through β -(1 \rightarrow 2) linkages. X-ray crystallography has indicated that the compound is amorphous.

Polyfructose can be produced from pure sucrose, sucrose in sugarcane or sugarbeet molasses or syrups, and from sugarcane or sugarbeet juice. The product made from molasses or juices requires some clean-up, for example, with DEAE cellulose, to attain the white color of the polyfructose made from pure sucrose. Gel permeation chromatography has shown polyfructose to have a narrow molecular weight range centered on 2×10^6 daltons.

The product is soluble in water, and is readily hydrolyzed at high temperatures and acid pH to fructose. Hydrolysis rates using several acids at various temperatures and the products formed, are presented. Polyfructose is not hygroscopic and can be stored at atmospheric conditions for several months. The compound is therefore an easily stored source material for fructose, which easily converts to fructose syrup upon acidification.

The products of enzyme hydrolysis of polyfructose are presented: the compound is resistant to most enzymes other than fructanases.

Potential applications of polyfructose include a sweetener source, encapsulation material and sweetness potentiator.

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Table 1.

Source of sucrose	% yield of levan, based on available fructose	% of original sucrose unreacted	Glucose formed, % of original sucrose
Beet Molasses	37	21	--
Beet Juice	28	23	8.5%
Cane Molasses	22	45	7
Cane Juice	57	38	20

Data after 2 days incubation.

Medium made to contain 8% sucrose, based on source.

Table 2. Acid hydrolysis to fructose.

	<u>TIME</u>	<u>TEMP.</u>	<u>% FRUCTOSE</u>
0.5% CITRIC ACID	48 hrs.	R.T.	--
	2 min.	microwave	33
	5 min.	microwave	76
	7 min.	microwave	100
0.5% ASCORBIC ACID	15 min.	100°C	100

Table 3. Hydrolysis of levans.*

TIME (min)	PERCENT FRUCTOSE	
	LOW MOL. WT. (20,000)	HIGH MOL. WT. (2×10^6)
0	0	0
10	29	24
20	68	46
30	96	68
40	100	88
60		92
80		100

*Hydrolyzed at 65 deg. C in 0.5% oxalic acid.

Table 4. Physical properties of polyfructose.

HIGH MOL. WT. (2×10^6 daltons)	$[\alpha]$ -42.3	M. Pt, °C >200
LOW MOL. WT. (20,000 daltons)	-62.2	92

Table 5. Applications and properties of polyfructose.

APPLICATIONS	PROPERTY
1. Source of sweetener syrup	Readily hydrolyzed
2. Sweetness potentiator	20,000 m. wt.
3. Storage compound for fructose	Non-hygroscopic
4. Thickener	Viscosity
5. Encapsulation of flavor	Flavorless unless hydrolyzed. Soluble
6. Encapsulation of color	Colorless, soluble
7. Encapsulation of pharmaceutical	Non-toxic, soluble
8. Tabletting	
9. Emulsifier	

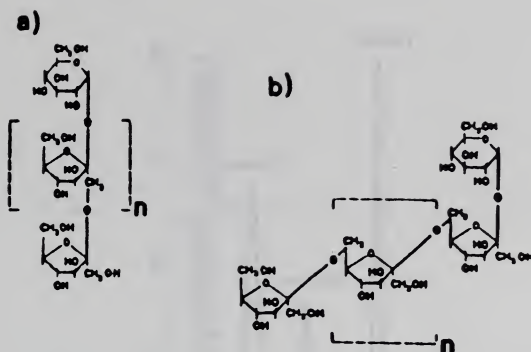


Figure 1. Different types of fructan. a) Inulin, β -(1-2);
b) levan, β -(2-6).

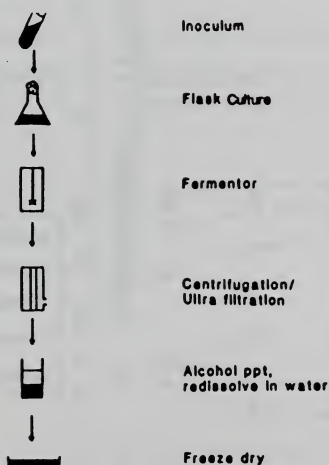


Figure 2. Scheme for production of levan by B. polymyxa.

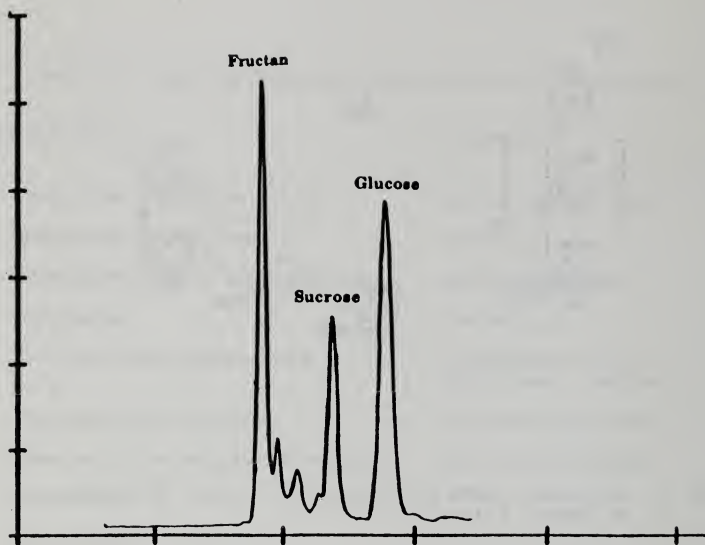


Figure 3. HPLC profile of microbial levan products at Day 2.

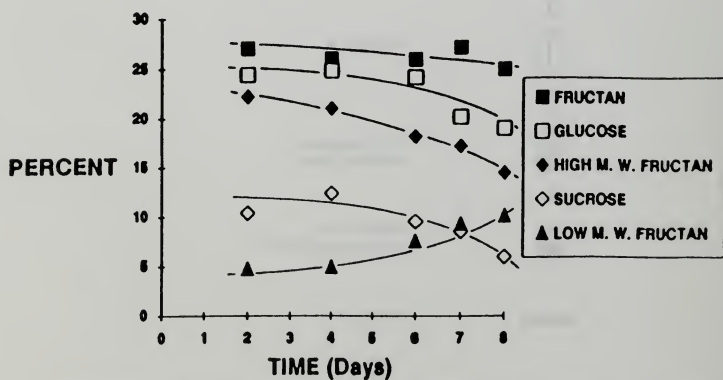


Figure 4. Composition of products from *B. polymyxa*.

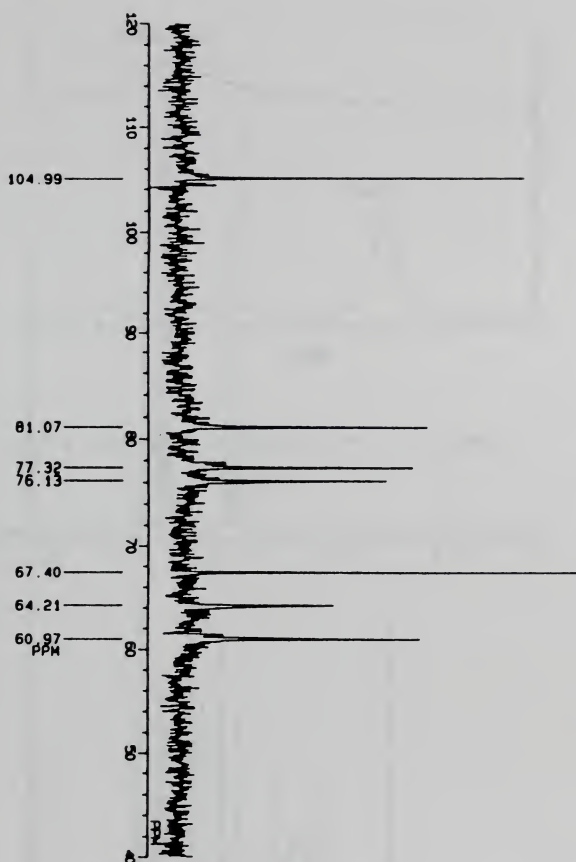


Figure 5. ^{13}C (nmr) 200 MHz (dioxane internal standard δ 67.40) of polyfructose.

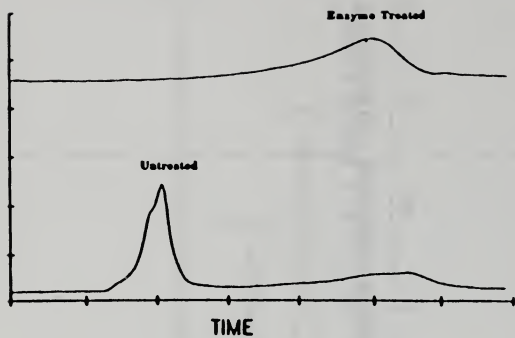


Figure 6. GPC profile of microbial levan.

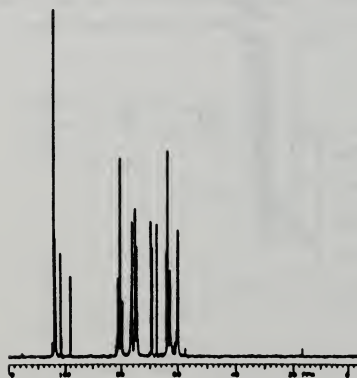


Figure 7. ^{13}C NMR spectrum of enzyme treated levan.

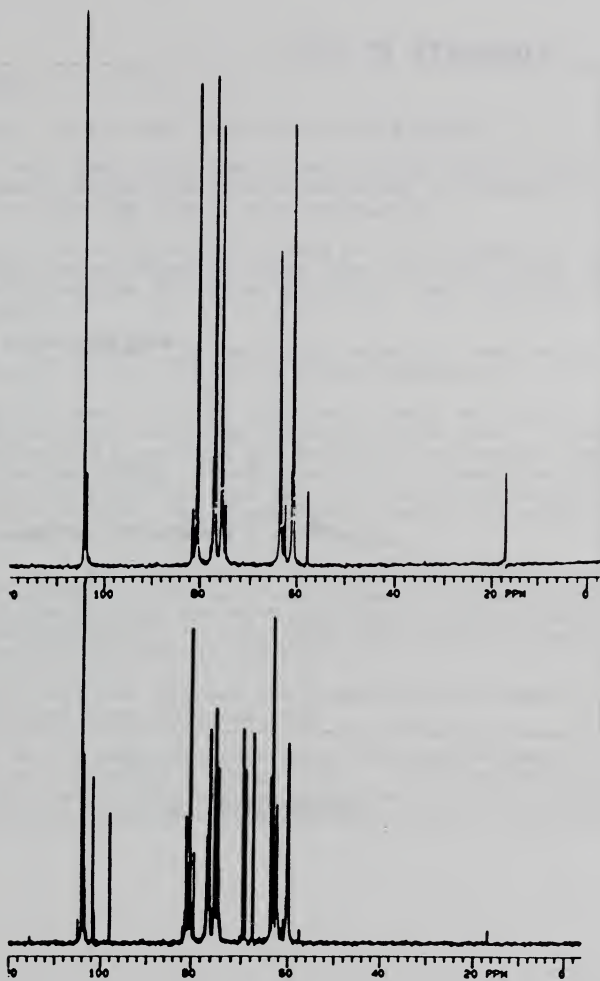


Figure 8. ^{13}C NMR spectrum of untreated and enzyme treated levan.

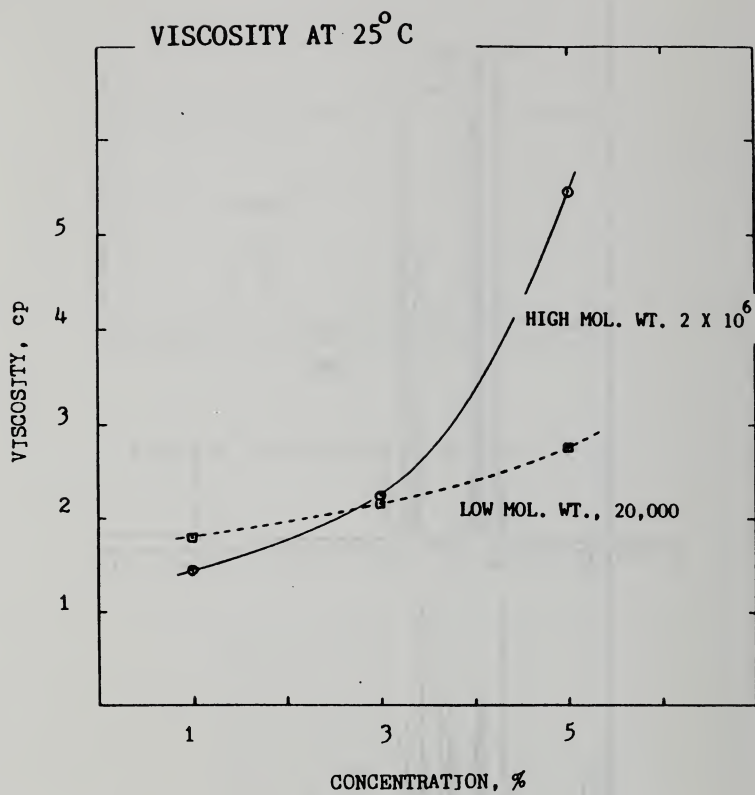


Figure 9. Viscosity profiles of high and low molecular weight polyfructose.

DISCUSSION

Question: Can you make the levans from glucose?

Bailey: No. That point was in the earlier report that I mentioned where we found that levan would not be produced from glucose or fructose with this microorganism.

Question: One of the ways we found we could produce levans is on whole sugar beets stored at about 70°F for long periods of time. That, unfortunately, is rather efficient. Have you tried whole beet tissue?

Bailey: No, not yet. We have used molasses. Beet molasses worked well. We are looking at liquid feedstocks.

Question: As you certainly will know, there is a long history of research on inulin and levan for more than 50 years. Many of the applications and properties you mention have also been known for a long time but at this moment there are no commercial applications. Do you think the improved yield of the polysaccharides will lead to economic application and compete with other products on the market with the same properties?

Bailey: Yes. As I mentioned, there are several microorganisms that can produce the levans from sucrose. However, B. polymyxa appears to be the only one that can produce such a high yield and at such a high purity. I think that this improved economics will stimulate more activity in the area.

Question: Have you improved the fermentation procedure? Have you tried continuous fermentation?

Bailey: No, we have concentrated on the chemical aspects rather than on the fermentation system itself. You mentioned past work - the fermentation systems have been worked on extensively but no high-yielding selective microorganisms was available before.

POLYSACCHARIDE AND COLORANT: A PROGRESS REPORT

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INTRODUCTION

For the last three years or so, S.P.R.I. has been publishing reports on high molecular weight colorants and polysaccharides in both cane and beet sugar manufacture (Godshall et al, 1987, 1988, Clarke, 1988). A colorant-polysaccharide complex has been proposed. The composition of the complex is different in beet and cane sugars, but both types of colorant-polysaccharide complexes come from the growing plant source. Colorant-polysaccharide complexes are large molecules that have low color compared to their molecular weight, but can travel through all processing steps, end up in crystal sugar, and have the potential to increase color in the final sugar product. In all cane refinery products, for example (Godshall, et al., 1982; Clarke, et al., 1988; Godshall, et al., 1988), a very high molecular weight (about 1×10^6) polysaccharide, with some color has been observed. (See Figures 1 & 2).

This paper will attempt to characterize the colorant-polysaccharide complex, and relate the impact of these compounds on processing efficiency and product quality. Recent work at S.P.R.I. in this area has focussed on tracing these complexes and their components through cane refinery and cane and beet factory processing. Results of these studies, as yet incomplete, are reported.

It should be emphasized that there are other categories of high molecular weight ($>10,000$ daltons) color. A 1987 study at S.P.R.I. (Godshall, et al., 1982) showed that about two-thirds (66%) of crystal color in affined raw cane sugar is over 20,000 daltons molecular weight. Most of this colorant, from 20,000 up to 1×10^6 daltons, does not have polysaccharide character and is very well removed in refining, as shown in Figure 1. Colorant at 1×10^6 daltons is not removed by refinery process, and is found in the white sugar crystal. This fraction of colorant contributes only a small proportion of color to the raw sugar, but, because it is preferentially occluded in crystallization, a much greater proportion of color to white sugar (see Figure 1). The cost of sugar manufacture is directly related to the cost of color removal. The degree of difficulty in removal of all high molecular weight color contributes to this cost. The colorant-polysaccharide complex passes through every process state, lowering the efficiency of each color removal process, and thereby increasing the cost of each process.

COLORANT-POLYSACCHARIDE COMPLEX

Sugarcane

These are two major possibilities for the structure of the colorant-polysaccharide complex. Both have been reported in monocot species of plants.

1. Cell wall polysaccharide (arabinoxylan type) with ferulic acid group attached by ester linkages. These compounds are known to exist in many plants of the Gramineae family, which includes wheat, corn, barley and sugarcane (Kato and Nevins, 1985; Harvey, et al., 1986). The cell wall polysaccharide is an integral part of the cell structure; the phenolic acid linked to it serves to connect to other cell structure; the phenolic acid linked to it serves to connect to other cell wall components, in some cases to lignin.

The chemistry involved in isolating and characterizing these complexes generally break down the large molecules that exist in the plant to smaller oligosaccharide units (3 to 6 sugar residues) that can be identified by chromatographic and spectrometric techniques, (methylation analysis by GC-MS, and n.m.r.). A typical fragment is shown in Figure 3. These fragments were isolated from bamboo (Ishi and Hiroi, 1990), but are typical of similar molecules isolated from other grasses (sugarcane is a giant grass). The phenolic acid shown here is the most frequently observed plant phenolic found in these complexes: ferulic acid.

Ferulic acid has been found as a component of sugar colorant (Meade and Chen, 1977). It is a pale yellow compound which has been shown, upon inclusion in sugar crystals, to cause rapid darkening and yellowing of the crystals (Devereux, 1986). Ferulic is not, however, the only phenolic compound that has been found in these cell wall complexes. Others include: coumaric acid, vanillin, p-hydroxybenzaldehyde and syringaldehyde (Hartley and Ford, 1989). The reader will recognize these compounds as all among those identified as cane sugar color components or precursors (Meade and Chen, 1977) by C.S.R.R.P.I. and S.P.R.I. Several of them, especially ferulic acid, can dimerize to a dehydroxyferulic acid. The dimer has more intense color at a higher wavelength in the visible range than the monomer. It is possible that ferulic (or another phenolic acid), carried into the sugar crystal by the polysaccharide complex, dimerizes as time passes (in storage) and causes color increase in the crystal.

There is no explanation at hand, however, for the release of ferulic acid from the complex (de-esterification), it is possible that sufficient energy is released by dimerization to break the ester linkage.

Ferulic acid-hemicellulose fragments (feruloylated trisaccharide, (0-5-0-feruloyl- α -L-arabino-furanosyl)-(1-3)-0- β -D-xylopyranosyl-(1-4)-D-xylopyranose) have been isolated from sugarcane bagasse, by Japanese workers (Fry, 1989; Roberts, et al., 1976). These fragments are similar to Compound A in Figure 3, where ferulic acid is esterified through the carboxyl group of an arabinose unit. While finding a compound in bagasse is not the same as finding it in sugar, the esterification of hemicellulose (itself fairly insoluble) will increase its solubility. Recent results on extraction of polysaccharides during sugarcane milling, shown late in this paper (Table 3) show extraction of cane polysaccharides with hot maceration water on bagasse. The compounds identified in bagasse are shown in Figure 4.

2. The second possible type of colorant-polysaccharide complex can come from the crosslinking of acidic polysaccharides with lignin (Fry, 1989). Part of the sugarcane cell wall material is an arabinogalactan with glucuronic acid units (glucuronoarabinogalactan) that has been given the trivial name ISP (Indigenous Sugarcane Polysaccharide) (Roberts, et al., 1976). I.S.P. is the type of acetic polysaccharide involved in lignin interaction. Rather less is known about this type of complex, though there is evidence for binding again through an ester linkage, in this case through the acid group on the polysaccharide. A type of acid polysaccharide interaction can also occur with small glycoproteins containing tyrosine or hydroxyproline. This last type of interaction may also occur in sugarbeet with pectin rather than glucuronans.

Sugarbeet

The sugarbeet, being a dicot, unlike the monocotyledonous grasses, has a different assembly of cell wall polysaccharides. Hemicelluloses in dicots are primarily xyloglucans, but recent work on sugarbeet polysaccharide has shown more arabinose than xylose, as shown in Table 1 (Clarke, et al., 1989; Broughton, et al., 1987). The acidic polysaccharides are pectins. Dr. J. F. Thibault, in an earlier paper at this conference, has described

sugarbeet pectins, and their reactions, in detail (Thibault, 1988), so only an outline will be included here.

Sugarbeet pectin is an acid polysaccharide with a backbone of rhamnose and galacturonic acid units. This rhamnogalacturonan backbone has side chains ending in esterified ferulic acid units (Thibault, 1988; Thibault, 1990). Although most pectin is removed in beet processing as calcium salts, it is quite likely that some pectin can continue through process and into the sugar crystal, carrying with it ferulic acid. The pectin material has a molecular weight range of 30-60,000 daltons. However, work at S.P.R.I. on high molecular weight colorant in beet sugar (Godshall, et al., 1989; Clarke, et al., 1989) shows that colorant of this size does not carry into white sugar (See Figure 2 and Table 2). There is a large proportion of this colorant in raw juice, thin juice and thick juice (See Figure 5), and some was observed in raw beet sugars, but it goes to molasses rather than into white sugar. Any of this type of colorant in white sugar is probably in the syrup coating the crystal. There is, however, colorant in white beet sugar of from 800,000 to 1×10^6 daltons. The higher molecular weight observed in molasses samples (higher than in sugar) is probably caused by the effect of higher ionic strength (in molasses) on the GPC column material. Thibault has shown (Thibault and Guillon, 1990; Thibault, 1990) that oxidative cross-linking of the ferulic acid-pectin material can increase gelation properties (turning a liability into a valuable asset), and also water absorptivity. Polymerized pectin moieties are a possible explanation for the very high molecular weight color, which is not observed in all white beet sugars. More likely in colorant is an esterified cell wall polysaccharide, similar to that in sugarcane, but probably esterified to ferulic acid through an arabinose, rather than the xylose common to other dicots. The new emphasis on work on beet cell polysaccharides (Clarke et al., 1989; Thibault, 1988) should give useful information to this end.

Experimental Techniques

This section will describe the systems used in this work, rather than list methods and materials.

The first major problem in studying high molecular weight (HMW) compounds is to separate it from low molecular weight material. The HMW compounds are present in well under 1% concentration in all substrates except molasses. Dialysis, using a rocking dialyzer, against flowing water, is still the most complete method of separation for 10g-100g samples. Various types of ultrafiltration, through membranes or in hollow fiber systems, can be used, but are expensive and give results that are difficult to reproduce for large samples.

The dialyzate obtained (after 4 days dialysis, concentration and freeze-drying) contains a mixture of all HMW colorants and polysaccharides over 12,000 daltons (the cut off of the dialysis column). Yield on a white sugar is usually below the 0.1% range. Further separation can be achieved on gel filtration columns; gel permeation chromatography, on Sephadex packing material chosen to separate compounds of molecular weight 10,000 to several million, has been the separation system of choice. Two detectors are used to measure both color intensity (U-V detector; 214 nm) and polysaccharide nature (refractive index detector) of fractions coming off the column.

Non-colored components can be separated from colored by a variety of resin or modified cellulose or other adsorbents; separations are slow because the high molecular weight compounds tend to adsorb onto column material.

Structure identification techniques include hydrolysis, with identification of hydrolysis products by HPLC, GLC or IC. Colorant compounds tend to breakdown during acid hydrolysis, and other color compounds can be formed from decomposition of component sugars as they are released by hydrolysis, so this technique is not useful for identification of colorant moieties. Methanolysis and acetolysis can give additional structural information, particularly for uronic acid moieties.

Methylation analysis with GLC and mass spectrometric quality of methylated fragments and nuclear magnetic resonance spectroscopy, especially carbon-13 nmr, are the major identification techniques when components have been separated.

In studies on factories and refineries, it has been found that high molecular weight material can most readily be monitored by following total polysaccharide concentration, especially for cane sugar manufacture. Small glycoproteins and small fractions of pectin can add to the low molecular weight polysaccharide component in sugarbeet processing. Dextran and starch can obfuscate the polysaccharide profiles, but an analytical scheme has been devised to separate them from cell-wall polysaccharides; this will be reported in a future publication.

Observations on process materials

Cane processing and refining

Figure 1 outlines the progress of high molecular weight colorant through a cane refinery. Only the very high molecular weight peak shows polysaccharide character. The large amount of colorant in the 100,000 dalton range is removed by refinery processes. Any remaining on white sugar is probably in the crystal coating.

It should, perhaps, be added here that when the colors of the various GPC fractions are summed up, the total is the same as the color of the initial sample. There are no artifacts (color created or removed) in the GPC separation.

Recent work on cane factory colorants, using similar experimental separation and characterization techniques, yields the gel permeation chromatograms shown in Figure 6, on clarified juice and raw sugar. The similarity of the raw sugar profile to the melt liquor profile in Figure 1 is noteworthy. The two raw sugars are not the same. The major difference in the profiles is the much smaller proportion of 30,000 dalton color in the melt liquor. It is proposed that this color is not preferentially occluded in crystallization; that the amount remaining in the raw sugar is mostly in the syrup coating, and is removed from the crystal in affination, leaving melted washed raw sugar with a proportionately lower amount of 30,000 dalton color than in whole crystal.

Isolation of the material of the very high molecular weight peak (1×10^6 daltons) in the clarified cane juice sample, and in the mixed juice sample that it came from, showed the following results:

	Peak at m.wt 1×10^6 daltons	
	Mixed juice (%)	Clarified juice (%)
arabinose	0.71	0.52
galactose	0.81	0.81
xylose	0.28	0.28
glucose	94.3	94.5
unknown	3.9	3.8

Most of the polysaccharide material is glucan, (dextran or starch); the remainder is cell wall polysaccharide: arabinoxylan, or glucuronoarabinogalactan.

Carbon-13 nmr analysis of this very high molecular weight material confirms that dextran is the major component, but indicates the presence of mixed polysaccharides (Keene and Clarke)). Further separation is in progress to obtain samples for definitive structural analysis.

Table 3 shows relative amounts of total polysaccharide (this includes starch and dextran) across several cane factories. The high levels of total polysaccharides in last roll juice, where hot water washes them out of the cane fiber, is noteworthy.

Studies are currently in progress to determine the character and origins of the 30,000 and 300-500,000 dalton colorants. Although

they are apparently removed to a great extent in refining, it will be valuable to know:

- (1) if they can be removed in raw sugar manufacture, and
- (2) which refining processes remove these most efficiently.

Beet processing

Figure 2 and Figure 4 outline the high molecular weight colorant in sugarbeet processing. Once again, it should be pointed out that the color of the individual fractions sums to the color of the whole sample, in each case.

The size groups are outlined in Table 2. Peak I, the very high molecular weight peak that remains in white sugar, has a pale yellow color. This is the peak that shows polysaccharide character, and is now proposed to be cell wall polysaccharide material, probably with ferulic acid or other phenolic acid groups esterified to it through a sugar linkage. The amount of ferulic acid is expected to be very small: Thibault (1990) has shown that in pectins there is less than 1%; less than that would be expected in this material.

Peak II, which is removed in process, is a pale buff color. This material is evidently removed to a great extent in carbonation, because thick juice contains proportionately less of it. Peak III, yellow in color, is thought to consist of pectins, which are also removed to a great extent in carbonation, and of polyphenolics reacted with aromatic amino acids (Broughton, et al., 1987; Madsen et al., 1978).

Current studies are examining the distribution of these three classes of colorant in between coating and washed crystal in white sugar, and also are following the progress of types of colorant through factory processing.

Table I. Composition (%) of beet polysaccharides (Vogel and Schiweck, 1988)

	SPRI (thin juice)	Südzucker (thin juice)
Arabinose	44.8	38.4
Galactose	34.1	28.5
Glucose	17.7	6.9
Xylose	2.4	0.2
Mannose	0.9	1.0

Table 2. Size groups (GPC peaks) of sugarbeet high molecular weight colorant

	I	II	III
White sugar	800,000		20,000
Raw sugar	1,000,000	300,000	20-30,000
Thick juice	1,000,000	200-400 slope	50,000
Molasses	800-1,000,000	200-300,000	30-50,000

Table 3. Polysaccharides extracted from sugarcane in milling

	A	B	C
Total polysaccharides, ppm:			
Crusher juice	2412	2608	5026
Last roll juice	7758	3560	10,778
Clarified juice	4322	2749	4323
Evaporator syrup	-	4566	2136
A-sugar	-	-	663

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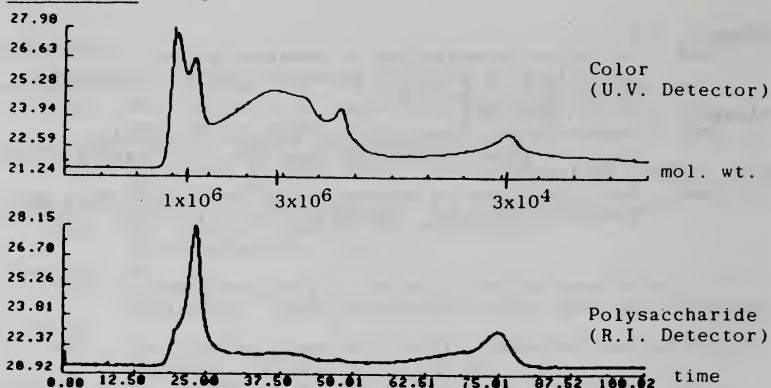
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MELT LIQUOR (1.0g)



FOURTH STRIKE SUGAR (0.8g)

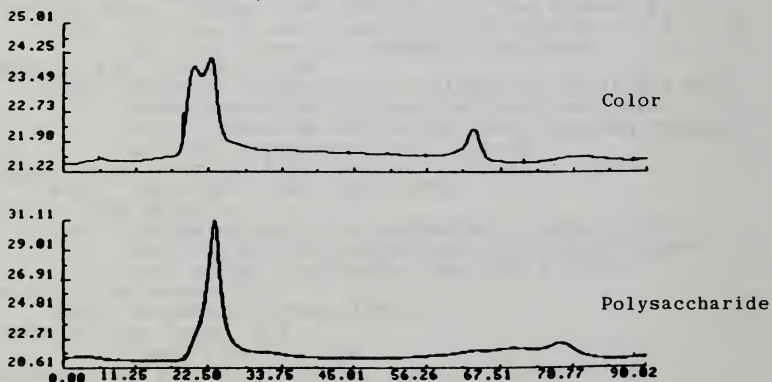
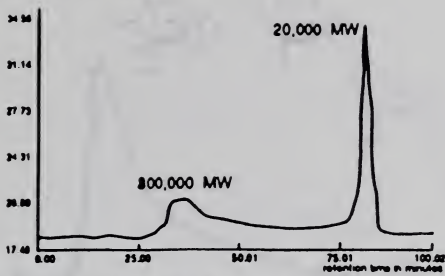
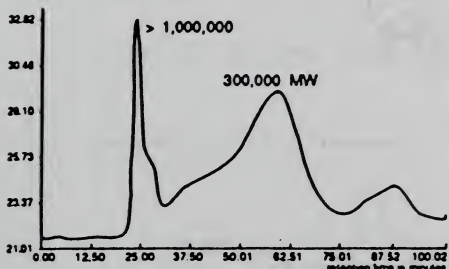


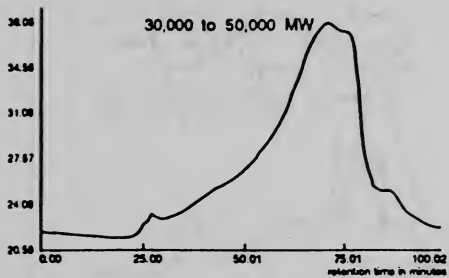
Fig. 1. GPC's showing color and polysaccharide nature of high molecular weight material from refinery samples



GPC profile of high molecular weight colorant from beet white sugar (25 g sugar)

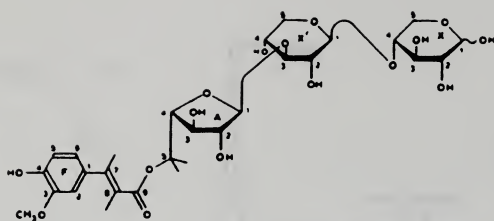


.. GPC profile of high molecular weight colorant from beet raw sugar (20 g sugar)

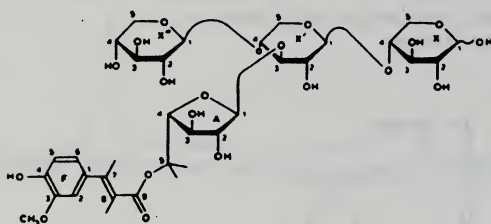


GPC profile of high molecular weight colorant from molasses (1g molasses)

Fig. 2. GPC of beet white sugar, beet raw sugar and molasses



Compound A



Compound B

Figure 3. Ferulic acid-hemicellulose fragments from bamboo cell wall polysaccharide

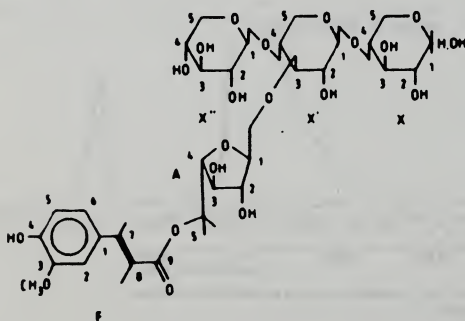


Figure 4. Ferulic acid-hemicellulose fragments from sugarcane bagasse cell wall polysaccharide

THICK JUICE (60g)

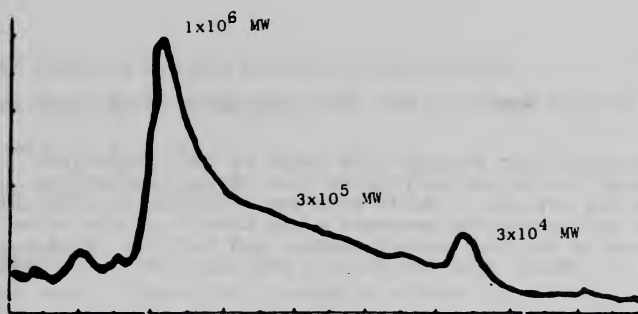


Fig. 5. GPC beet thick juice

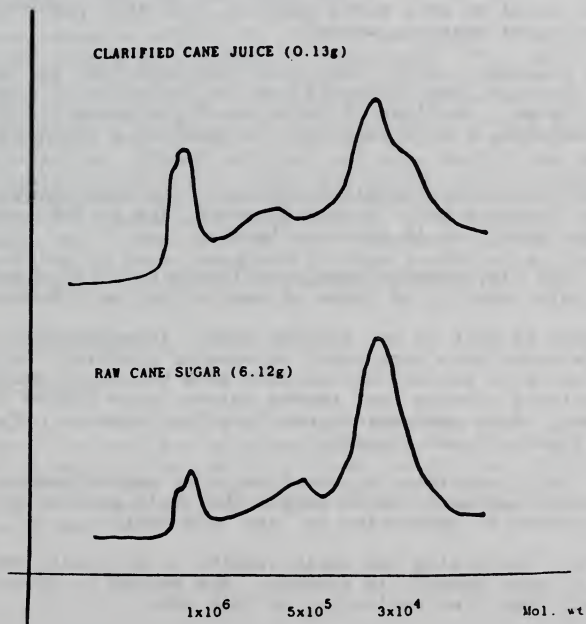


Fig. 6. GPC of clarified cane juice and raw cane sugar

DISCUSSION

Question: Can you expand on how these compounds cause color in storage?

Clarke: We think that ferulic acid could be one responsible factor, though how it can be removed from its polysaccharide backbone in the crystal, I don't know yet. Ferulic acid esterified onto a polysaccharide presents a way that a soluble color precursor can be carried through process and into the crystal. Perhaps we can obtain some feruloylase and learn more about these molecules.

Question: Can you say how much of the color of white beet sugar at, say, 20 ICUMSA units, can arise from these hemicellulose complexes, and how much from other materials.

Clarke: I can only speculate. The condition of the sugar-fresh or stored - is important. Perhaps 10% or so of the color in a fresh sugar could be from these sources, with that percentage increasing as the sugar is stored.

Question: Yesterday, Prof. Mantovani explained that he thought the color in white sugar crystals was the inclusion of droplets of mother liquor. In light of this, would you speculate on why these polysaccharide materials are preferentially occluded in the crystal?

Clarke: They are very soluble molecules, with sugar groups on their ends. Perhaps Prof. Mantovani would like to try some of our colorant fractions in his experiments.

Question: The big question from a refiner's point of view is how to handle this material in terms of decolorization processes.

Clarke: That is part of our current work. It's difficult to do large scale tests with such small amounts of material. Since the hemicellulose-type polysaccharide have acid groups, I would think the decolorizing systems that remove anions could remove that, but obviously these systems are not totally effective. Those answers are part of our program.

Question: Is it possible to hydrolyze these polysaccharide - colorants with enzymes? Would making the chain smaller make them easier to remove by adsorption on, say bone char?

Clarke: Yes, decreasing the chain length, or molecular weight should help their removal in process. The enzyme to choose is another ball game - we're looking at that now.

INVERT ANALYSIS WITHOUT WET LEAD CLARIFICATION

C. Chi Chou and S. H. Mercene

Presented by W. Buescher

Amstar Sugar Corporation

INTRODUCTION

Amstar Sugar Corporation's standard method for determining invert in process streams calls for the use of wet lead as clarifying agent prior to analysis. It was established many years ago that, without lead clarification, the reproducibility among analysts was not acceptable. In view of the governmental restrictions with respect to hazardous waste landfill disposal, this study was conducted to evaluate various non-hazardous reagents as a potential replacement for the lead reagent.

The present accepted method for routine analysis of reducing sugar is the Lane-Eynon volumetric procedure. This method is based upon the reduction of the copper (II) complex with tartaric acid in alkaline solutions. However, preparation of the sample prior to analysis may vary. The classic way of using lead clarification and subsequent de-leading is still used in Amstar Sugar Corporation. ICUMSA and Tate and Lyle Analytical Committee found irregular results with lead, and abandoned the method in favor of removal of lime salts by EDTA and potassium oxalate (Schneider, 1979; Meade and Chen, 1977). In this study, both Amstar Sugar and ICUMSA methods were evaluated and the results were correlated to those obtained by High Performance Liquid Chromatography (HPLC) and Ion Chromatography (IC).

MATERIALS AND METHODS

Lane-Eynon Method

Sample solutions were prepared of such strength that they contained from 0.25 to 0.8 g of reducing sugars per 100 ml., then defecated or decalcified, using various reagents as shown in Table 1, and filtered. Filtrate was analyzed for invert using the Lane-Eynon method as described in the ICUMSA procedure except for sample preparation.

Table 1.--Amount of different reagents used per gram of sample

a)	Lead subacetate	1 ml
b)	EDTA (4%)	4 ml
c)	Potassium oxalate (5%)	2.4 ml
d)	Ecosorb S402	2-3 g

a)	sodium oxalate added to solution until precipitation ceases, then diluted to mark.	
b,c)	as per ICUMSA recommendation	
d)	stirred for 10 min., 3-4 g filter cell #1 added, stirred for another min. and filtered.	

HPLC

Analyses were carried out with (A) Waters Associates' Sugar Analyzer I liquid chromatograph model SAI equipped with, 401 refractive index detector, M45 solvent delivery system with reference value and Eldex column heater, (B) a 710B WISP Waters automatic sampler, (C) a 730 Waters data module with LC calculation capability, and (D) a Bio-Rad Aminex Carbohydrate HPX-87 column of 300mm x 7.8mm operated at 85°C with a flow rate of 0.6ml/min. The eluent used was water originating from a Millipore Milli-R/Q water purification system, containing 20mg/L Calcium propionate added and filtered with 0.45 micron pore size membrane.

Ion Chromatography

Dionex 4000i IC system equipped with pulsed amperometric detector I and Spectro-Physics 4270 computing integrator was used. Samples were introduced via Dionex injector, through a Carbo Pac column with the mobile phase of 0.16N NaOH at 1 ml/min flow rate.

RESULTS AND DISCUSSION

Lane-Eynon reducing sugars using different clarifying agents

Several samples of high and low purity process streams were treated with lead, EDTA and potassium oxalate prior to invert analysis. For comparison purposes, analysis of untreated samples were also performed. Results are set out in Table 2. When compared to the decalcification with EDTA or potassium oxalate, defecation with lead with subsequent de-leading gives similar results for high purity samples and about 2% lower results for low purity samples. This difference could be ascribed to the

lead precipitation of other reducing substances that appear to be more abundant in low purity samples. McDonald proved conclusively by chromatographic methods that this lead precipitate contained no dextrose or levulose but did contain material that reduces Fehling solution. Lead clarification also increased the sharpness of the end point in titration with methylene blue because of the resulting clear solution. As shown in Table 2, % invert for samples without any reagent are similar those with EDTA or potassium oxalate added.

Lane-Eynon reducing sugars using neutral lead acetate and Ecosorb S402 as clarifying agents

Besides EDTA and potassium oxalate, several other reagents were tried as potential replacement for lead. As shown in Table 3, Ecosorb S402 appears to be the best choice as the replacement. Ecosorb S402 is a powdered activated carbon based industrial precoat product, with styrene divinyl anion exchange resin in chloride form as an active material. It decolorized process streams samples as effectively as lead, and with the aid of the filter cell, filtered slightly faster (Table 5).

Lane-Eynon using lead and Ecosorb S402 vs. HPLC and IC

The reducing sugars in high and low purity process stream as determined by HPLC and IC were found to compare well with the reducing sugars obtained by the conventional Lane-Eynon method, using lead and Ecosorb S402 clarification, as evidenced by the results shown in Table 5. A comprehensive work was done by Wnukowski comparing HPLC with Lane-Eynon using lead defecation and he came up with a relatively good agreement between the two methods. He further noted that HPLC values tend to be lower as the purity of sample decreases. This deviation may have been caused by incomplete precipitation of other reducing substances thus making Lane-Eynon results a little higher. As can also be observed in Table 4, Ecosorb results is in closer agreement with HPLC and IC as compared to lead.

CONCLUSION

This study has established that Ecosorb S402 can be used to replace lead reagent in clarifying sample solution for invert analysis. The use of hazardous lead reagent has been discontinued as of May 1990 in accordance with governmental regulations.

It was also found that Lane-Eynon determination of invert using lead clarification gives results closest to the true invert value as determined by HPLC and IC methods. All other reagents as recommended by ICUMSA gives results higher than the "true" value.

Table 2.--% Reducing sugar by Lane-Eynon method using different clarifying agents

Sample	Clarifying Agents			
	Lead Acetate	Disodium EDTA	Potassium Oxalate	Nothing Added
Melted Finals	2.40	2.86	2.80	2.80
Melted Finals	3.64	4.42	4.46	4.26
High Raw Green Syrup	9.48	11.24	11.23	11.22
Medium Raw Green Syrup	9.93	12.25	12.40	12.10
Remelt Syrup	11.91	13.89	13.86	13.59
High Remelt	12.81	14.00	13.91	13.95
Blackstrap Molasses	10.08	12.27	12.16	12.16
Blackstrap Molasses	11.08	13.21	13.29	12.92
Blackstrap Molasses	17.16	19.40	19.32	18.98
Blackstrap Molasses	10.17	12.19	12.30	11.86

Table 3.--Comparison of Lane-Eynon reducing sugars using neutral lead acetate and Ecosorb S402 as clarifying agents.

Sample	% Invert		
	Neutral Lead Acetate	Ecosorb S402	diff.
Melted Finals	2.74	2.56	- 0.18
High Remelt	5.04	5.09	0.05
Medium Green	9.83	9.80	- 0.03
Medium Raw Green	7.47	7.53	0.06
High Raw Green	8.58	8.89	0.31
Remelt Syrup	10.53	10.01	- 0.52
Remelt Syrup	11.34	11.40	0.06
Blackstrap Molasses	9.92	9.85	- 0.07
Blackstrap Molasses	12.27	12.37	0.10
Blackstrap Molasses	12.16	11.95	- 0.21
Blackstrap Molasses	16.25	15.38	- 0.86

Table 4.--Comparison of % invert in process streams by Lane-Eynon using lead and Ecosorb S402 as clarifying agents, HPLC and IC methods.

Sample	Lane-Eynon		HPLC	IC
	with Lead	with Ecosorb	un-clarified	un-clarified
Melted Finals	2.40%	2.43%	2.02%	2.15%
High Remelt	7.87%	7.95%	7.94%	7.82%
High Remelt	9.47%	9.26%	9.37%	9.11%
Blackstrap Molasses	10.57%	-	9.21%	8.78%
Blackstrap Molasses	12.68%	12.92%	12.88%	13.96%
Blackstrap Molasses	17.58%	16.53%	15.51%	14.38%

Table 5.--Filtration time in minutes (average of 10 samples)

Neutral lead acetate (without filter aide)	22 min
Ecosorb S402 (with filter aid)	20 min

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DISCUSSION

Question: Many thanks for an interesting paper. We also are searching for clarification agents for sugar factory juices, in our case for the polarization determination. Have you done some tests of your clarification procedure also with polarization samples?

Buescher: No, we haven't tried that yet, this is a relatively recent procedure. We've stopped using lead at all in our refineries. We got away from it by using a short cell-length polarimeter. For raw sugar analysis, we use the calcium hydroxide-aluminum chloride clarification (Ref: Clarke and Legendre, Proc. S.I.T., 1989: 219-239).

Editor's note: The use of powdered carbon resin materials as a substitute for lead acetate in clarification for polarisation analysis was tried at SPRI in 1988, and found to give irregular and irreproducible results on polarisation. The conclusion at that time was that the reagent removed non-reproducible amounts of sucrose. Because invert level are so much lower than sucrose levels, presumably the error caused by the reagent is much lower (low enough to be non-significant) in the invert analysis described here.

BALANCE OF CATIONS AND ANIONS IN SUGAR BEET PROCESSING.

P.W. van der Poel, J.M. de Bruijn, N.H.M. de Visser
and J. Konings

CSM Suiker bv

The fundamentals of juice purification have clearly been described by Dedek, Brieghel-Müller, McGinnis and others. These authors have drawn the attention towards electrochemical balances of cations and anions in the juice purification operations.

Progress in analytical chemistry enables us nowadays to establish balances for cations and anions by rapid and not too complicated procedures.

Analysis of cations like sodium, potassium, calcium and magnesium have been state of the art since many years. Real progress has been made as far as concerns the analysis of juice anions by HPLC-methods, i.e. Ion Moderated Partition and Ion Chromatography. The closing entry of the balance carbonic acid can be estimated by gas sensitive electrode. This is also the case for ammonia. Balances of cations and anions are available now as a tool for management for beet quality studies, process optimization and trouble shooting.

The paper reviews the work which has been done in CSM on the subject and reflects the state of the art on the basis of some applications in practice.

INTRODUCTION

Evaluation of the efficiency of the juice purification in a sugar factory is a problem as such. A university standard is hard to define. Chemistry of juice purification is related to many chemical reactions in which some components are eliminated by precipitation and others are degraded into various reaction products. The final result is a thin juice or a second carbonation juice, the quality aspects of which are related to white sugar yield and quality.

Thin juice quality is determined by a large variety of components. In many of the cases the chemistry of the individual components is known, so that we can predict their behaviour under the conditions of the juice purification process. Thin juice quality parameters, lime salts and thermostability are affected by chemical reactions during beet storage, extraction and juice purification as well as by the quality of the beet. Lime salts content and thermostability of second carbonation juices may be considered as the closing entries of the electrochemical cation/anion balances (van der Poel et al., 1988).

When I had the pleasure to meet professor Dedek with my friends of the Tirlmont Refinery he summarized very clearly: "Effective alkalinity is bicarbonate content." Dr. McGinnis characterizes second carbonation chemistry in his book (Beet Sugar Technology) on page 253. "This unit process is remarkable for its simplicity of purpose and complexity of chemistry" (McGinnis, 1982). Modern analytical chemistry however, offers the possibility to lift the curtain of complexity and has applied by CSM to prepare decisions such as:

- What will be the influence of ammonia recycling on juice purification and environment.
- What will bring the application of magnesium oxide as it is applied in the Magox process (Schoenrock, 1974) or magnesium carbonate as proposed in the Methathesis process (Chadwick, 1983).
- What is the efficiency of soda ash for decalcification and for increase of thermostability.
- Can we predict molasses formation from beet analysis with an acceptable accuracy.

Our studies concerned the course of individual non-sugars in the factory process as well as combined electrochemical balances of cations and anions.

CATIONS FROM BEET TO MOLASSES

Sodium and Potassium

Sodium and potassium salts as molassigenic components have been followed by CSM for more than 30 years. The interest arose from the beet quality studies in the early sixties (Asselbergs et al., 1960 and 1963). The results of the investigation were also used in process optimization (Blok et al., 1976).

In our factory routine we follow potassium and sodium from beet to molasses. The sources of potassium and sodium are:

- the beet material,
- the soda ash supply,
- the decalcification plants.

The part of the sodium and potassium, which is eliminated with the exhausted cosettes, depends on the sugar losses to the pulp and the eventual use of pressing agents. This has been subject of pilot plant studies (van der Poel et al., 1973).

Beet Analysis

At the CSM's beet receptions we analyse the beet samples for sugar content, tare, potassium, sodium and α -amino N. In the 963 campaign we analysed for the first time beet samples on K and Na on full tare house scale.

Table 1 records an example derived from one of our weekly reports of the 1989 campaign.

Table 1. Beet quality (camp. 1989, week 3)

Factory	Vierverlaten	Halfweg	Breda
* number of samples	10900	3871	7265
* % analysed for K, Na, α -amino N	99.8	98	99
* potassium mmol/kg	50.2	53.5	44.8
stand. dev.	6.5	7.6	7.5
* sodium mmol/kg	6.2	7.6	7.7
stand. dev.	2.4	3.3	3.9
* α -amino N mmol/kg	21.3	21.8	21.2
stand. dev.	6.0	5.4	6.8
* K+Na mmol/kg	56.4	61.1	52.5
stand. dev.	7.5	8.3	8.9
* ratio $\frac{\text{mol sucrose}}{\text{mol (K+Na)}}$ in molasses	0.87	0.82	0.87

The frequency distributions are recorded in Figure 1 (page 347). The figures are reported to the growers and used by the agricultural staff in their growers advisory programs.

Extraction of sodium and potassium for beet cossettes

From composite samples of exhausted cossettes together with the beet analysis, we can derive the partition of potassium, sodium and α -amino N between raw juice and pulp. Table 2 gives the figures.

Figure 1. Frequency distributions tare house samples

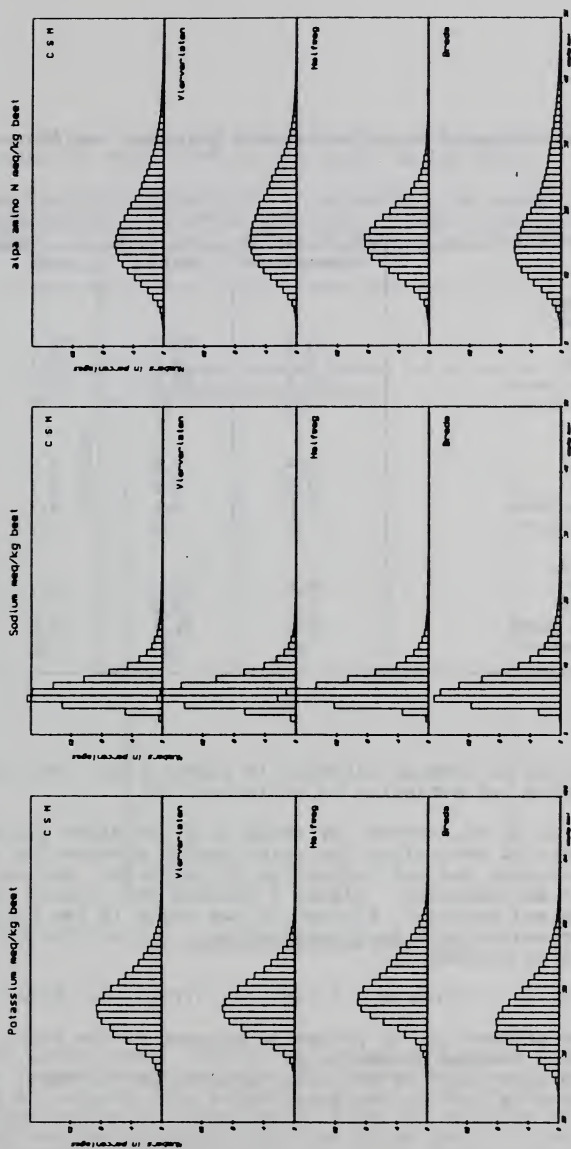


Table 2. Partition of K and Na between pulp and raw juice

Factory	Vierverlaten	Halfweg	Breda
<u>meq/kg beet</u>			
Potassium:			
in beet	47.0	50.5	46.0
in pulp	7.7	4.6	7.1
in raw juice	39.3	45.8	38.8
% extracted	84	91	84
Sodium:			
in beet	6.4	7.0	7.5
in pulp	1.4	0.9	1.7
in raw juice	5.0	6.1	5.7
% extracted	77	87	77
α -amino N:			
in beet	24.7	22.3	24.7
in pulp	1.1	0.6	1.9
in raw juice	23.6	21.8	22.8
% extracted	95	97	92

The extraction of α -amino nitrogen is almost 100%. The extraction of sodium and potassium is influenced by:

- the extraction of sucrose. By means of pilot plant extraction experiments we determined the relationship between the extraction of sucrose and the extraction of potassium, sodium, α -amino N and chloride. Figure 2 records the figures for potassium and sucrose. Plotted on the abscis is the percentage of the potassium left in the cossettes. Curve fittings gave a third degree polynomial.

$Y + 0,1425 x^3 - 2,1196 x^2 + 9,9252 x + 0,7255$, in which

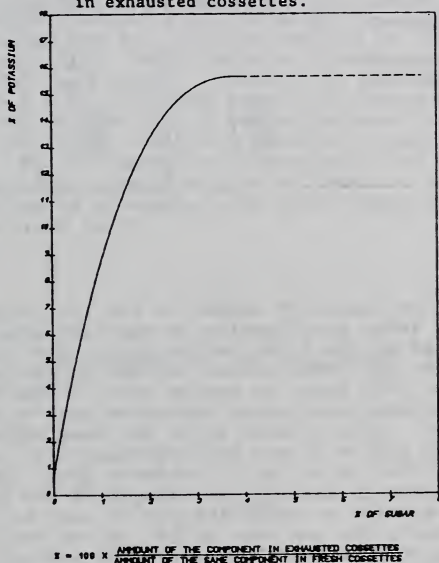
* Y = the percentages of potassium present in the beet, which remains in the pulp.

* x = the percentage of the sugar present in the beet which is left in the pulp

The correlation is valid for sugar losses lower than 0.4% on beet which is about 2.5% of the sugar in the beet.

Knowing the ratio sugar/K+Na in molasses, the sugar losses to molasses can be related to the sugar losses in exhausted cossettes. This is part of our diffusion optimization program.

Figure 2. Relation between sucrose and potassium in exhausted cossettes.



- Another factor which influences the extraction of potassium and sodium from the beet material is the use of lime salts as agents to improve pulp pressing. Comparison of the extraction with application of these agents with the standard situation without the use of these additives is an input in decision making concerning the feasibility of the application of these agents.

Potassium and sodium balances from beet to thick juice

Additional sources of sodium after the extraction are: supply of soda ash or caustic soda and ion exchange in the decalcification plants. The check in the accuracy of the balances is done by the analysis of thick juices. Table 3 records an example.

Table 3. Balances of K + Na from beet to thick juice.

Factory	Vierverlaten	Halfweg	Breda
meq K+Na / kg beet:			
in raw juice	46.3	51.0	46.1
from decalcification		1.6	2.7
from soda ash		0.3	1.2
from caustic soda	7.6		
Sum	53.9	52.9	50.0
from thick juice	53.6	54.9	53.7
analysis			

Magnesium

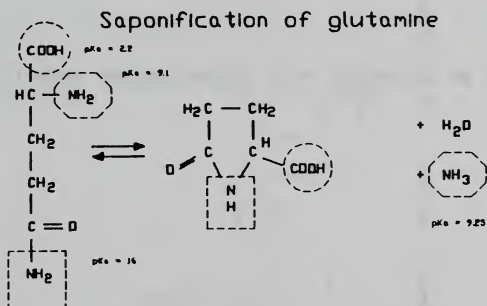
Magnesium is another important cation in the raw juice. It is eliminated in the juice purification as the hydroxide is almost insoluble at the pH of the first carbonation. The removal of magnesium decreases the total cation content of the second carbonation juices with about 18 meq/kg beet. This decreases in its turn the carbonate/bicarbonate concentrations in the second carbonation juices. As magnesium salts are less molassigenic than potassium and sodium it may be advantageous to re-introduce magnesium as the oxide or as the bicarbonate after the first carbonation. We mention the work of Schoenrock and Chadwich. In this way magnesium oxide or carbonate can be used to increase the bicarbonate and carbonate contents of the second carbonation juices and consequently reduce the lime salts content. The efficiency depends on the level of lime salts at which the process is applied. We will come back to this subject further in this paper.

Calcium

Calcium exists in relatively low concentrations in the raw juice. The calcium contents of the second carbonation juice may be considered as the closing entry of the electro-chemical balance on the cation side. The solubility of the lime salts is controlled by the concentration of the CO_3^{2-} ion.

The course of ammonia from beet to thick juice

We refer to our studies which we presented at the XVth General Assembly of CITS (van der Poel, 1981). The ammonia concentrations in raw juices are low. The main source is the condensate which is used as diffuser supply water. Ammonia is in the NH_4^+ form at the pH in the extraction plants. The main part of the ammonia in the diffuser supply water is absorbed by the exhausted cossettes by ion exchange reactions of NH_4^+ ions against K^+ and Na^+ ions. pH correction by sulphitation or addition of other acids is necessary. During main liming, carbonation and in the clarifiers, saponification of amides takes place. In one series of samples we determined the percentage of α -amino N which was sensitive to saponification with formation of ammonia. In this case the percentage was 45%. This percentage is not a constant for every beet quality. From the amide fraction about 60% is saponified during main liming. Roughly, $24 \text{ meq/kg beet } \alpha\text{-amino N}$ gave: $24 \times 0.45 \times 0.60 = 6.5 \text{ meq ammonia in juice purification}$. At the pH and the temperature of second carbonation, this corresponds to 1.4 meq of NH_4^+ cations. When in the equilibrium $\text{NH}_4^+ \rightleftharpoons \text{NH}_3 + \text{H}^+$, NH_3 is removed by evaporation, the equilibrium proceeds to the right supplying protons from the NH_4^+ cations. The saponification of glutamine into pyrrolidone carboxylic acid and ammonia is given below.

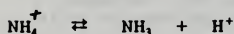


In this case the functional groups for the base acid equilibrium on the right and the left side are not very different. Saponification of glutamine is not of great influence on the cation/anion balance as long as NH_3 -loss by evaporation is prevented. In the evaporators where NH_3 is removed from the juice, the result of the reaction becomes manifest. During evaporation, saponification of the residual glutamine and asparagine proceeds; the ammonia is distilled off, leaving as many protons in the juice as there are NH_4^+ ions.

Thin juice has more ammonia in the cation NH_4^+ form than second carbonation juice due to the pH decrease caused by sulphitation.

We now come back to the ammonia in the practical examples of juice decalcification and stripping of ammonia from juices.

Here we give the general equilibrium:



$$\frac{[\text{NH}_3]}{[\text{NH}_4^+]} \frac{[\text{H}^+]}{[\text{H}^+]} = 10^{-9.4} \text{ at } 20^\circ\text{C} \text{ and about } 10^{-7.6} \text{ at } 85^\circ\text{C}$$

(see Appendix 1)

Amino acids

Amino acids play an important role in the buffer capacities of the juices.

Ammonia steam-stripping plant in a beet-sugar factory (design)

150 kg NH₃/ha



COURSE OF THE MOST IMPORTANT ANIONS FROM RAW JUICE TO MOLASSES

Table 4 records the composition of a raw juice and the corresponding second carbonatation juice.

Table. 4. Anions and cations in factory juices.

	meq/kg beet Raw juice	meq/kg beet 2nd Carb. juice
Citrate	15.6	2.7
Malate	3.8	2.7
Oxalate	10.3	0.2
Phosphate	6.2	<0.1
Sulphate	4.1	2.5
Sulphite	3.1	<0.1
Formate	2.9	3.7
Acetate	2.1	4.1
Lactate	1.2	6.8
Pyrrolidone Carb.	1.0	7.8
Chloride	4.4	4.4
Nitrate	4.4	4.3
K	40.0	40.0
Na	5.5	6.7
Ca	2.2	2.0
Mg	18.0	0.4
Cu	<0.1	<0.1
Fe	0.5	<0.1

We divide the anions into 5 categories.

Anions with completely soluble lime salts, such as: chloride, nitrate, nitrite, lactate, acetate and amino acids. These acids are not eliminated in juice purification.

Acids with slightly soluble lime salts, such as phosphate, sulphate, oxalate, citrate and malate. Major proportions of these anions are precipitated in juice purification.

Acids originating from degradation of invert sugar, such as lactic, formic, acetic and saccharinic acids. Although saccharinic acids can be analysed by HPLC, their concentrations are not given in Table 4 as we did not have available the pure compounds required for calibration.

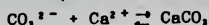
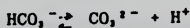
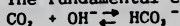
The lime salts of these acids are soluble. The acids are formed during juice purification.

Pyrrolidone carboxylic acid and aspartic acid are formed by saponification of the amides. These acids are partly counter-balanced by NH_4^+ as a cation in second carbonation juices.

Carbonic acid

Bicarbonate and carbonate concentrations are low in raw juice. In second carbonation juice however, these anions form the closing entry on the anion balance, which then equalizes the sum of the anions to the sum of the cations in order to maintain the electro-chemical equilibrium between cations and anions. Bicarbonate is in equilibrium with carbonate which determines the solubility of calcium.

The fundamental equations are:



The corresponding equilibria are:

$$\frac{[\text{CO}_3^{2-}] \cdot [\text{H}^+]}{[\text{HCO}_3^-]} = K_{\text{HCO}_3^-}$$

$$[\text{CO}_3^{2-}] \cdot [\text{Ca}^{2+}] = K_{\text{sp-CaCO}_3}$$

THE SOLUBILITY PRODUCT OF CALCIUM CARBONATE

It is clear from professor Dedek's work, that it is not justified to consider the solubility product of calcium carbonate in sugar juices as a physical constant (Dedek et al., 1966). The value depends on the composition of the juices. The activities of the Ca^{2+} ions are influenced by sugar and many juice components. He mentions for instance the complexing properties of ammonia and citrate. Brieghel-Müller determined the solubility product of calcium carbonate in pure sugar solutions. He found a value of $0.75 \cdot 10^{-6}$ ($T=85^\circ\text{C}$) (Brieghel-Müller et al., 1953). Dedek gives for aragonite solutions in water at 80°C a value of $\text{p}K_{\text{sp-CaCO}_3} = 8.75$ which gives a solubility product of $0.18 \cdot 10^{-8}$ (Dedek et al., 1966). We determined in second carbonation juices the lime salts and total carbonic acid contents. From the carbonic acid contents, the pH and the temperatures, we calculated the CO_3^{2-} concentrations by means of the $\text{p}K_{\text{HCO}_3^-}$ - value of 9.93 (85°C). We refer to the work which we presented at the 16th General Meeting of CITS (Appendix 2). For the juices in question we can calculate a simplified solubility product from the carbonate and lime salts contents. For this purpose second carbonation juices were heated in closed bottles with pure calcium carbonate

to reach equilibrium. Temperature was 85°C and contact time 30 minutes. Total carbonic acid content and lime salts were determined after filtration. Table 5 gives the figures.

Table 5. "Solubility product" of calcium carbonate in factory second carbonation juices.

pH 20°C	CaO mg/100 ml	CO ₂ ppm	Ca ²⁺ mol/l 10 ⁻³	CO ₃ ²⁻ mol/l 10 ⁻⁴	solubility product (85°C) 10 ⁻⁷
9.18	5.20	193	0.93	0.78	0.72
9.42	7.30	113	1.30	0.79	1.03
9.45	7.00	123	1.25	0.93	1.15

ADDITION OF AMMONIA TO FIRST CARBONATION JUICES TO INCREASE THE CARBONATE CONTENT.

Recycling of ammonia would give a possibility to control the carbonate contents and thus the lime salts contents in second carbonation. The procedure would also reduce the environmental pollution and ammonia. The investigations were in three parts:

- laboratory experiments,
- full scale factory experiments with addition of ammonia in the first carbonation juices after filtration,
- design of an industrial plant for ammonia recycling and feasibility study as far as concerns the costs and benefits.

Laboratory studies

Second carbonation with addition of ammonia. The figures are given in table 6.

Table 6. Second carbonation with ammonia addition.

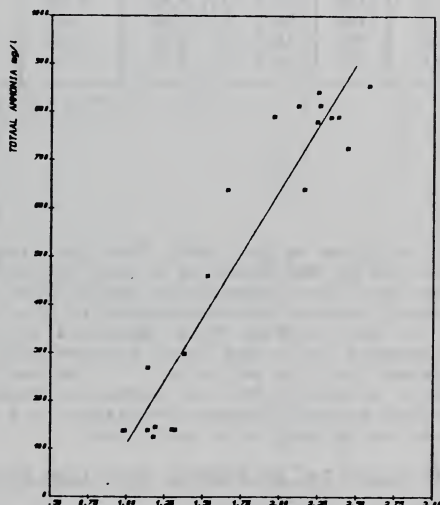
pH	total ammonia mg/l	CaO mg/100ml	CO ₂ mg/l	Ca ²⁺ mol/l 10 ⁻³	CO ₃ ²⁻ mol/l 10 ⁻⁴	solubility product 10 ⁻⁷
9.22	269	3.70	390	0.66	1.78	1.14
9.17	460	3.30	660	0.59	2.61	1.53
9.23	640	3.30	810	0.59	3.67	2.16
9.20	813	2.60	1080	0.46	4.57	2.12

There is a slight increase in the solubility product at increasing ammonia concentrations. This is in agreement with the literature (van der Poel et al., 1973). We did not make corrections for activities. This would lead us too far beyond the scope of this presentation.

Full scale factory studies

Ammonia was added to the first carbonation juice after filtration for a period of about 3 hours. Samples of second carbonation juice after filtration were taken every 20 minutes before and during the ammonia addition. The figures are recorded in table 7. Figure 3. illustrates the influence of ammonia on the solubility product.

Figure 3. Relationship solubility vs. ammonia content



solubility product $\times 10^7$

The results are in agreement with the laboratory studies. The solubility products are of the same order and the influence of increased ammonia concentrations on the solubility of the calcium carbonate appears to be reproducible. A value of 1.10^{-7} seems to be applicable for normal factory juices.

Table 7.

pH	NH ₃ mg/l	CaO mg/100ml	CO ₂ mg/l	CO ₃ ²⁻ %	Ca ²⁺ mol / 10 ⁻³	CO ₃ ²⁻ mol / 10 ⁻³	solubility product 10 ⁻⁷
9.15	138	6.8	258	1.4	1.21	0.82	0.99
9.15	138	6.7	258	1.4	1.20	0.82	0.98
9.20	141	6.6	254	1.9	1.18	1.10	1.30
9.25	140	6.7	232	2.1	1.20	1.10	1.32
9.25	139	6.1	219	2.1	1.09	1.05	1.14
9.25	125	5.8	236	2.1	1.04	1.13	1.18
9.25	146	5.8	239	2.1	1.04	1.14	1.19
9.40	502	4.7	589	2.9	0.84	3.88	3.26
9.30	725	2.9	900	2.3	0.52	4.70	2.44
9.25	789	2.6	1063	2.1	0.46	5.07	2.33
9.20	780	2.7	1063	1.9	0.49	4.59	2.24
9.20	790	2.3	1104	1.9	0.41	4.77	1.96
9.20	814	2.6	1139	1.9	0.46	4.92	2.26
9.20	814	2.6	1138	1.9	0.46	4.91	2.26
9.20	790	2.8	1100	1.9	0.50	4.75	2.38
9.20	842	2.6	1135	1.9	0.46	4.90	2.25
9.25	855	2.8	1104	2.1	0.49	5.27	2.58
9.15	638	3.0	968	1.4	0.54	3.08	1.66
9.05	298	4.6	570	1.3	0.82	1.68	1.38

From the process point of view we conclude, that the lime salts contents of the juices can be decreased to a very low level by addition of ammonia to the first carbonation juice after filtration. As we have already ammonia concentrations in the thin juices up to 140 ppm, we can increase this concentration by recirculation of the ammonia until the level reaches about 800 ppm. This would decrease the lime salts to 2 - 3 mg CaO/100 ml which would enable us to eliminate the ion exchanger decalcification plants. The ammonia surplus becomes available in a concentrated solution, which can be used as a fertilizer.

Design and feasibility study for an ammonia recycling plant.

We have in principle two possibilities to recycle the ammonia. By distillation from the thin juice or by distillation from the condensate of the evaporators. An outline of the equipment and its incorporation in a beet sugar factory is given in Figure 4. The design is for a slice capacity of 125 tonnes/hr with an ammonia input of 28 kg/hr and 120 kg/hr ammonia in circulation (mass flows 1 and 13 in Figure 4 and Table 8). With this equipment we may expect lime salts contents of the thin juice of 2.5 mg CaO/100 ml which makes decalcification needless. The mass flows are recorded in Table 8.

Figure 4

Armenia steam-stripping plant in a beet-sugar factory (design)

Mass-flow figures refer to table 7

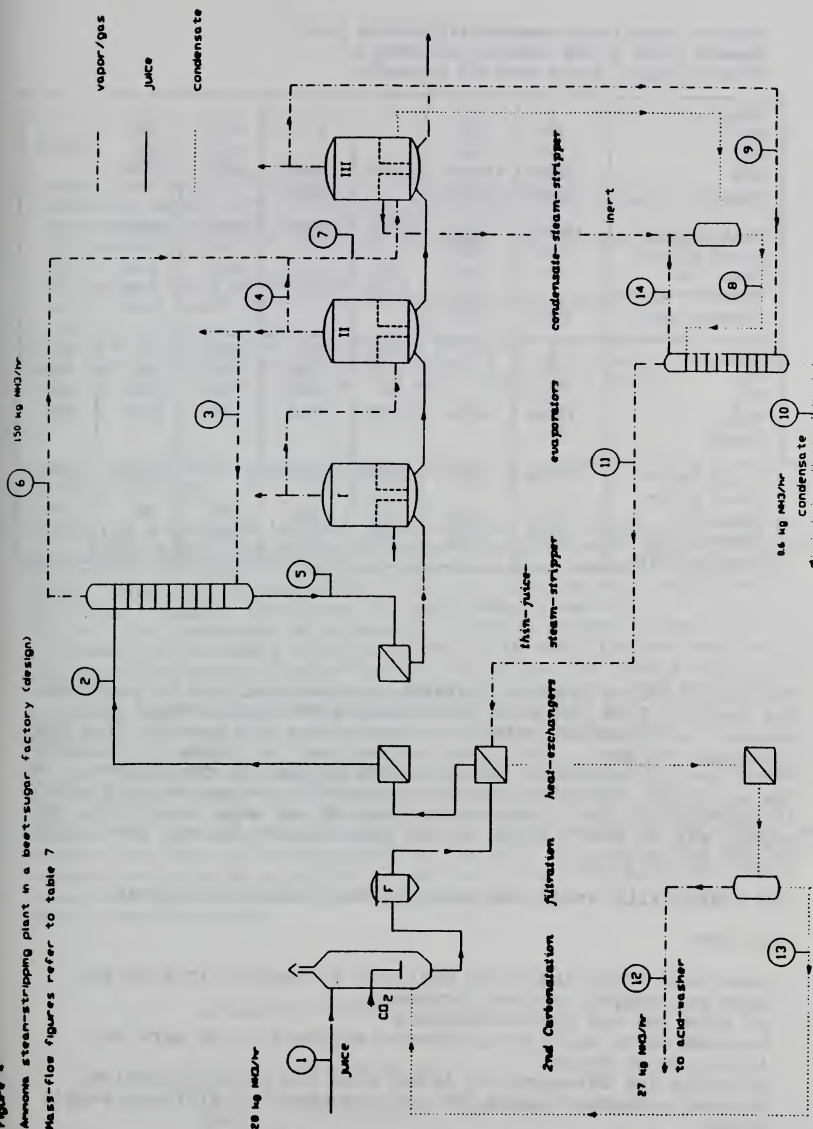


Table 8. Mass flows ammonia distillation plant.
Numbers refer to the numbers in figure 4.
Flows in kg/hr, slice rate 125 tonnes/hr.

Flow no.	1	2	3	5	6	7	
NH ₃	28	150		0.3	150	150	
CO ₂	56	162		-	162	162	
H ₂ O	160000	161000	18000	163000	18850	18850	
Sugar	26000	26000		26000			
Total kg/hr	186000	187000	18000	189000	19162	19162	
Total m ³ /hr	177	182					
Temp. °C	95	120	122	123	120	120	
Pressure kg/cm ²		2.0	2.16	2.15	2.0	2.0	
Density kg/m ³	1050	1030					

Flow no.	8	9	10	11	12	13	14
NH ₃	103		0.6	149	27	122	47
CO ₂	43		0	162	56	106	119
H ₂ O	18000	1050	17900	2000	43	1950	792
Sugar							
Total kg/hr	18150	1050	17900	2311		2178	958
Total m ³ /hr							
Temp. °C	116	110	109	102	85	85	116
Pressure kg/cm ²	2.0	1.46	1.4	1.2	1.2	1.2	2.0
Density kg/m ³							

The installation has two distillation columns; one to eliminate the ammonia from the thin juice before the evaporation and another much smaller column to concentrate the ammonia from the condensed vapours. The first column uses 18 tonnes of steam/hr which can be integrated in the steam balance of the factory. As the enthalphy decrease in the distillation column is very small it corresponds to a temperature drop of not more than 2°C. The vapour may be used in the vacuum pan house or in the third body of the evaporators.

The feasibility study was based up the following aspects:

Benefits:

- decreased sugar losses to molasses as decalcification and soda ash supply are not necessary,
- no soda ash and brine necessary,
- reduced water purification costs as there is no more NH₃ in the condensates,
- no costs for discharge of brine from the decalcification,
- reduced amounts of acid for pH adjustment of diffuser supply water,
- positive synergy with other aspects of the environmental program.

Costs:

- amortization of the plant,
- need for supply of alkalising agents to the evaporators; MgO seems the most appropriate,
- increased amount of sugar to molasses due to the addition of MgO,
- increased fuel consumption,
- additional heat surface in evaporators necessary.

The total balance for the project was positive, but the returns were not such that the project earned a high priority. The pay-back time of the project was in order of 6 to 7 years.

USE OF ALKALISING AGENTS SUCH AS SODA ASH OR MAGNESIUM OXIDE FOR DECALCIFICATION.

Magnesium carbonate is much more soluble than calcium carbonate, this makes magnesium oxide and magnesium carbonate applicable as sources of cations to increase the dissolved amount of carbonic acid. The case is comparable with the ammonia recirculation. The alkali supply increases the bicarbonate contents of the juices. An increased bicarbonate contents of the juices. An increased bicarbonate concentration is in equilibrium with an increased carbonate concentration, which in its turn reduces the solubility of the lime salts. From the previous work we might conclude that a solubility product $[Ca^{2+}][CO_3^{2-}] = 1.10^{-7}$ is a useful value for factory juices of normal composition.

We will estimate the efficiency of alkali supply for a thin juice with (for European conditions) high lime salts contents (17 mg CaO/100 ml). In this example we take MgO as alkalising agent. We estimate the amount of MgO which would be necessary to reduce the lime salts contents to its half by increasing the CO_3^{2-} concentration with a factor 2. We can also calculate the efficiency of the procedure in terms of millimoles CaO removed per millimole MgO added.

We use the following figures:

$$[Ca^{2+}][CO_3^{2-}] = 1 \times 10^{-7}$$

$$pH = 9.2 \text{ measured at } 20^\circ C$$

$$\frac{[CO_3^{2-}]}{[HCO_3^-]} = 0.019 \text{ at } 85^\circ C \text{ (see Appendix 2)}$$

The calculated amount of MgO be considered as the minimum value. In practice it may be necessary to choose a lower pH in second carbonation than 9.2 to increase the solubility of the magnesium oxide. We based our estimations on complete dissolution and dissociation of MgO. If we have to decrease the pH for instance to 8.5 to dissolve the MgO, the ratio

$$\frac{[\text{CO}_3^{2-}]}{[\text{HCO}_3^-]}$$

decreases from 0.019 to 0.0053, which means a considerable reduction of the efficiency of the MgO as deliming agent. The procedure has its own optimal pH-value, which is lower than the normal value of about 9.2.

Thin juice lime salts content 17 mg/100 ml

$$17 \text{ mg/100 ml} = 170/56 = 3.04 \times 10^{-3} \text{ mol/l}$$

$$[\text{Ca}^{2+}] [\text{CO}_3^{2-}] = 1 \times 10^{-7} \quad [\text{CO}_3^{2-}] = \frac{10^{-7}}{3.04 \times 10^{-3}} = 0.33 \times 10^{-4} \text{ mol/l}$$

$$\frac{[\text{CO}_3^{2-}]}{[\text{HCO}_3^-]} = 0.019$$

$$[\text{HCO}_3^-] = \frac{[\text{CO}_3^{2-}]}{0.019} = \frac{0.33 \times 10^{-4}}{0.019} = 1.74 \times 10^{-3} \text{ mol/l}$$

In order to double the HCO_3^- content and consequently the CO_3^{2-} concentration, we need an equivalent amount of MgO which is $1.74/2 = 0.87 \text{ mmol/l}$. This is to obtain the desired carbonate concentration. For precipitation of 85 mg/l CaO we need an equivalent amount of MgO. This means $85/56 = 1.52 \text{ mmol/l}$. So, to eliminate 1.52 mmol/l CaO, we need $0.87 + 1.52 = 2.39 \text{ mmol MgO}$. The efficiency of the procedure is in this case.

$$\frac{1.52}{2.39} = 64\%$$

For a daily slice capacity of 10.000 tons beet and a thin juice draft of 130% this means a minimum daily use of 1150 kg pure MgO to bring a lime salts content of 17 mg/100 ml down to 8.5 mg/100 ml.

The MgO use in practice will be larger due to the solubility of MgO at pH 9.2

Estimation of the efficiency of alkali addition for decalcification.

We calculated the amount of alkali which is necessary to decrease the lime salts contents with 2 mg CaO/100 ml. We started with lime salts contents between 20 mg CaO/ml and 4 mg CaO/100 ml. Table 9 records the figures.

Table 9. Efficiency of alkali addition in relation to lime salts content, based upon a decrease in lime salts content of 2 mg CaO/100 ml.

concentrations in mol x 10 ⁻⁴	starting lime salts content mg/100 ml				
	20	16	12	8	4
CaO concentration	35.7	28.5	21.4	14.3	7.1
equilibrium [CO ₃ ²⁻]	0.28	0.35	0.47	0.70	1.40
equilibrium [HCO ₃ ⁻]	14.74	18.42	24.74	36.84	74.21
equivalents [CO ₃ ²⁻] + [HCO ₃ ⁻]	15.30	19.12	25.68	38.24	77.03
<hr/>					
[CO ₃ ²⁻] at 2 mg/100 ml lower lime salts cont.	0.31	0.40	0.56	0.93	2.80
[HCO ₃ ⁻]	16.32	21.05	29.47	49.12	147.37
equivalents [CO ₃ ²⁻] + [HCO ₃ ⁻]	16.94	21.85	30.59	50.98	152.97
[CO ₃ ²⁻] supply	1.64	2.73	4.91	12.74	75.94
alkali supply to replace removed CaO meq/l. 10 ⁻⁴	7.14	7.14	7.14	7.14	7.14
total alkali supply meq/l. 10 ⁻⁴	8.83	9.87	12.05	19.88	83.08
yield	81%	72%	59%	36%	9%

Remark

The calculations are valid for soda ash and caustic soda. The efficiency of the MgO will be lower in practice than in the given calculations, as the availability of the alkali from MgO at pH 9.2 is not 100%.

Conclusion

The process is efficient to eliminate lime salts from juices of poor quality. It is not an alternative for decalcification if lime salts contents of 2 à 3 mg/100 ml are desired.

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APPENDIX 1

Ratio $\text{NH}_3/\text{NH}_4^+$ for 20°C and 85°C based on the pKa values of ammonia. Extrapolations make the figures at 85°C less reliable than the figures at 20°C.

Ratio $\text{NH}_3/\text{NH}_4^+$ as a function of the pH.

pH 20°C	$\frac{\text{NH}_3}{\text{NH}_4^+}$	% NH_4^+	pH 85°C	$\frac{\text{NH}_3}{\text{NH}_4^+}$	% NH_4^+
12.0	398	0.2	11.0	2398	0.0
11.0	39.8	2.4	10.0	240	0.4
10.0	3.98	20.0	9.0	24.0	4.0
9.5	1.25	44.2	8.5	7.59	11.6
9.4	1.00	50.0	8.4	6.03	14.2
9.3	0.79	55.7	8.3	4.79	17.2
9.2	0.63	61.3	8.2	3.80	20.8
9.1	0.50	66.6	8.1	3.02	24.8
9.0	0.40	71.5	8.0	2.40	29.4
8.9	0.32	75.9	7.9	1.91	34.4
8.8	0.25	79.9	7.85	1.70	37.1
8.7	0.20	83.3	7.75	1.35	42.6
8.6	0.16	86.3	7.7	1.20	45.4
8.5	0.126	88.8	7.65	1.07	48.2
8.4	0.100	90.9	7.5	0.96	51.1
8.3	0.079	92.6	7.55	0.85	54.0
8.2	0.063	94.0	7.5	0.76	56.8
8.1	0.050	95.2	7.5	0.68	59.6
7.9	0.032	96.9	7.4	0.60	62.4
7.8	0.025	97.5	7.35	0.54	65.0
7.7	0.0199	98.0	7.3	0.48	67.6
7.6	0.0158	98.4	7.25	0.43	70.0
7.5	0.0126	98.7	7.2	0.38	72.4
7.4	0.0100	99.0	7.15	0.34	74.6
7.3	0.0079	99.2	7.1	0.30	76.8
7.2	0.0630	99.3	7.05	0.22	82.3
7.1	0.0050	99.5	7.05	0.22	82.3
7.0	0.0040	99.6	7.0	0.24	80.6

At the pH of the second carbonation, 9.2 at 20°C which corresponds to ~8.2 at 85°C 29% of the ammonia is in the NH_4^+ form, 80% is in the NH_3 form and does not contribute to the cation balance.

Ratio $\frac{\text{CO}_3^{2-}}{\text{HCO}_3^-}$ as a function of the pH

pH 20°C	$\frac{\text{CO}_3^{2-}}{\text{HCO}_3^-}$	% HCO_3^-	pH 85°C	$\frac{\text{CO}_3^{2-}}{\text{HCO}_3^-}$	% HCO_3^-
12.0	224	0.4	11.0	11.7	7.8
11.0	22.4	4.2	10.0	1.17	45.9
10.0	2.24	30.8	9.0	0.117	89.4
9.5	0.71	58.5	8.5	0.037	96.4
9.4	0.56	64.0	8.4	0.029	97.1
9.3	0.45	69.1	8.3	0.023	97.7
9.2	0.35	73.8	8.2	0.019	98.1
9.1	0.28	78.0	8.1	0.0148	98.5
9.0	0.22	81.7	8.0	0.0117	98.8
8.9	0.18	84.9	7.9	0.0093	99.0
8.8	0.141	87.6	7.85	0.0080	99.1
8.7	0.112	89.9	7.75	0.0070	99.3
8.6	0.089	91.8	7.7	0.0059	99.4
8.5	0.071	93.3	7.65	0.0053	99.4
8.4	0.056	94.6	7.6	0.0047	99.5
8.3	0.045	95.7	7.55	0.0042	99.6
8.2	0.035	96.5	7.5	0.0037	99.6
8.1	0.028	97.2	7.5	0.0037	99.7
8.0	0.022	97.8	7.45	0.0034	99.7
7.9	0.0177	98.2	7.4	0.0030	99.7
7.8	0.0141	98.6	7.35	0.0026	99.7
7.7	0.0112	98.8	7.3	0.0023	99.8
7.6	0.0089	99.1	7.25	0.0021	99.8
7.5	0.0071	99.2	7.2	0.0019	99.8
7.4	0.0056	99.4	7.15	0.0017	99.8
7.3	0.0045	99.5	7.1	0.0015	99.8
7.2	0.0035	99.6	7.05	0.0013	99.8
7.0	0.0022	99.7	7.0	0.0012	99.8

DISCUSSION

Question: Thank you for a very good paper, really getting into the fundamentals of juice purification. You said indications were that use of a pulp pressing aid, for example calcium sulfate, actually increased the sodium content of the juice. I assume you are referring to a weak cation exchange between calcium and sodium within the pulp matrix.

van der Poel: Yes, Calcium against sodium and potassium. In practice, this can be followed by determining the percentages of potassium and sodium which remain in the pulp.

Question: This is rather interesting in reference to the work on alkaline diffusion. Would you suggest that the same thing could apply there - that the addition of lime or saccharate milk to cossettes would increase the extraction of sodium and/or potassium?

van der Poel: Yes, I think that's one of the things we must consider in making feasibility studies of alkaline extraction.

Question: When you run this process, in order to keep up the pH in second carbonation with ammonia, do you run first carbonation, in such a way as to keep the ammonia in?

van der Poel: Yes. Of course, these were experiments that needed a lot of preparation. We added ammonia to first carbonation after filtration. In that case, of course, you have to stop the decalcification plant. Otherwise, the decalcification plant will take the ammonia from the juice and replace it with sodium. You have to change the amounts of acid you have to add to the diffuser, because you could get a lot more ammonia in the condensate. This was an experiment just as addition, without the distillation columns, to obtain the data. We ran it for about 3 hours, and increased the ammonia concentration in the factory's juice and took samples every 20 minutes, and so could follow the lime salts levels.

Question: On the other end, in second carbonation we expect some color adsorption. Is there any change in color adsorption when you run second carbonation at a higher pH?

van der Poel: We did not run a higher pH. We kept the pH in second carbonation the same. I want to note that, if magnesium oxide is applied, then the pH must be decreased.

ION CHROMATOGRAPHIC DETERMINATION OF RAFFINOSE IN WHITE SUGAR

W.S. Charles Tsang, G.-L.R. Cargel, and M.A. Clarke

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Raffinose, a trisaccharide (galactosyl sucrose), is present in sugarbeets in significant amounts, particularly in beets which have subjected to prolonged cold temperature. Since it is chemically stable under processing conditions and does not break down, it tends to accumulate in the beet molasses.

Raffinose influences the crystallization rate of sucrose by exerting a depressing effect. Large amounts of raffinose cause the sucrose crystals to elongate along the B-axis and assume needle-like shape. Raffinose also affects the polarization determination for sucrose which may lead to sugars polarizing at over 100%, that is, at falsely high pol.

Practically, it is important to be able to measure the concentration of raffinose to better control crystallization, to enhance recovery in the sugar end, and to determine if the use of alpha-galactosidase (melibiase) enzyme is necessary. Raffinose in a series of beet molasses samples had been studied by HPLC, using three different systems and the results were compared with those obtained by an enzymatic method (Tsang et al., 1987). The amino-bonded silane system using acetonitrile-water as the mobile phase has been shown to give the most accurate analysis for raffinose (Proc. ICUMSA, 1982; Reinefeld et al., 1988).

Following the success in the trace analysis of invert in raw and white sugar using a Dionex ion chromatographic system (Tsang et al., 1989 and 1990), the level of raffinose present in white cane and beet sugars was examined. The first part of this paper will relate results obtained from the determination of trace levels of raffinose in white sugars and molasses. The paper will also describe the analysis of a series of soft drinks of various origins in an attempt to identify the type of sugar used by the manufacturers.

EXPERIMENTAL

Materials

Raffinose pentahydrate (J. T. Baker Co.)
Sodium hydroxide solution, 50% W/W (Fisher Scientific Co.)
Sodium acetate, HPLC grade (Fisher Scientific Co.)

Methods

IC Analysis: Ion chromatography was conducted on a Dionex model 4500i series IC system equipped with pulsed amperometric detector and Dionex 4400 electronic integrator. Samples were introduced via a Rheodyne injector with a 25 μ l loop. A CarboPac PA1 anion exchange column was used at ambient temperature with a flow rate of 1 ml/min. Two solvent systems were used: a) For raffinose - 30 mM NaOAc and 100 mM NaOH and b) For sucrose, maltose, raffinose, kestoses and nystose - 40 mM NaOAc and 100 mM NaOH.

Standard - 10 ppm raffinose (or 11.78 ppm raffinose pentahydrate)

Sample concentration: 5000 mg/l for white sugar; 1000 mg/l for beet and cane molasses; 5% solution for soft drink

IC conditions: Sensitivity 3K E_{APP} : $E_1 = 0.01$, $E_2 = 0.65$, $E_3 = -0.90$

RESULTS AND DISCUSSIONS

With the advance of modern chromatographic technique, rapid and direct IC determination of glucose and fructose in raw and refined sugars has been shown to be possible (Tsang et al., 1987; Tsang et al., 1990). Results indicated that IC analyses compare well with the more established GLC analyses (Tsang, 1989). IC can also be used successfully for detection of organic anions at low levels (Tunland, 1988).

Mantovani and Vaccari (1964) found a significant amount of raffinose in crystalline sucrose obtained in solutions containing raffinose and concluded that raffinose is adsorbed on the surface of the growing sucrose crystals and finally becomes trapped in the crystals. Initially, the plan was to measure directly, by IC, the trace level of raffinose present in white beet sugar.

In Fig. 1A (18,000 mg/l white beet sugar), a small peak, which was later identified as raffinose, emerges after the big sucrose peak whereas no such peak is observed in Fig. 1b (18,000 mg/l white cane sugar). To get an accurate analysis of the raffinose content in white beet sugar, various concentrations (from 1800 to 18,000 mg/l with an increment of 1800 mg/l) of white beet sugar samples were analyzed (Fig.2); and the results are given in Table I.

Table I. Content of raffinose in white beet sugar measured at different concentrations by IC

Sugar conc.	ppm raffinose	ppm raffinose
<u>mg/l</u>	<u>detected</u>	<u>on solid</u>
1800	1.58	878
3600	3.00	833
5400	4.44	822
7200	5.96	828
9000	7.03	781
10800	8.47	784
12600	9.40	746
14400	9.07	630
16200	7.61	470
18000	7.40	411

From the data in Table I, it is clear that the level of raffinose measured begins to decrease when the concentration of the white beet sugar used is greater than 9000 mg/l. The optimum concentration for white beet sugar (without overloading the column) is in the range of 1800-7200 mg/l. The concentration of white beet sugar chosen for injection 5000 mg/l. The identity of the raffinose peak is established in the following manners: a) spiking of the white beet sugar with raffinose causing the raffinose to co-elute with the small peak following the big sucrose peak, and b) addition of 4 mg/l raffinose to 5000 mg/l white cane sugar (contains no raffinose) causing the resulting mixture to show an elution profile identical to that obtained for white beet sugar (Fig. 3).

A total of 20 white beet sugar samples were analyzed for raffinose by this IC method. The results are summarized in Table II and III. All of the white beet sugars in Table III except two originated in Europe.

Table II

Determination of raffinose level in white beet sugars (domestic) by IC technique

<u>White Beet Sugar</u>	<u>ppm Raffinose</u>
Domestic 1	806
2	970
3	498
4	718

Table III. Determination of raffinose level in white beet sugar (Non U.S.) by IC Technique

<u>White Beet Sugar</u>	<u>ppm Raffinose</u>
Non-U.S. 1	1116
2	793
3	773
4	779
5	667
6	726
7	774
8	633
9	395
10	428
11	768
12	449
13	965
14	341
15	644
16	854

No raffinose peak was observed when white cane sugar samples (10) were analyzed. In one case, a minute amount of raffinose was detected which may result from the mixing of raw cane sugar with raw beet sugar by the refinery. This refinery is known to have occasionally mixed its inputs. Similarly, raffinose was not observed in a sweet sorghum sugar sample (Fig. 4).

This technique is applied to identify the origin (from beet or cane) of the granulated sugar in sugar packets which were collected recently from various places and results are given in Table IV.

Table IV. Identification of raffinose present in granulated sugar

<u>Source of Sugar Packets</u>	<u>Presence of Raffinose</u>
Hotel, West Germany	Yes
Sugar Company, Ireland	Yes
Hotel, Victoria, B.C.	No
Hotel, Seattle	Yes
Hotel, Boston	Yes
Sugar Company, Louisiana	No
British Airways	Yes

Thus, this IC method can differentiate between the white beet and cane sugars and may be useful for the detection of adulteration in fruit juice (Copola, 1984). Adulteration by addition of corn or cane sugar to apple juice can easily be detected by a technique called Stable Isotope Ratio Analysis (SIRA). Adulteration with beet sugar, however, cannot be detected by SIRA alone.

Soft Drink Analysis

This rapid and simple analytical method can also be applied to determine the type of sweetener used in carbonated soft drinks. Almost every major soft drink manufacturer in the United States now uses high fructose corn syrup (HFCS) to sweeten soft drinks. Starch derived sweeteners show traces of maltose, isomaltose and other starch breakdown products that are not present in cane or beet sugars, and in invert syrups from cane or beet. A chromatogram of a recently purchased sample of cola (5% solution), which was sweetened with HFCS, is shown in Fig. 5a. Under the chromatographic conditions used in the analysis of cola drinks, isomaltose (5.33 min) elutes after the big glucose-fructose invert peak and is followed by maltose (13.00 min). A series of unidentified peaks, which is characteristic of starch-derived glucose syrup, appears between isomaltose and maltose. Further dilution of the cola drink (0.25%) leads to the separation of glucose and fructose (Fig. 5b). At this level only isomaltose and maltose are detected.

Of the three brands of soft drink manufactured and purchased in Canada (sugar and/or glucose on a label), all tested contained glucose, fructose, sucrose, maltose and glucose residues (Fig. 6a). This would indicate that a mixture of sucrose and glucose syrup was used in the soft drinks. The appearance of a maltose peak accompanied with a slight rise and fall of the baseline points to the presence of glucose syrup. In some cases, it is rather difficult to assign the sweeteners used by the manufacturers, that is, to distinguish between glucose from sucrose and glucose from corn starch. There is no conspicuous maltose peak in Fig. 6b; however, the elution profile suggests a mixture of sugar and glucose syrup was used.

Figure 7 illustrates a chromatogram of lime-lemon drink which was sweetened by white beet sugar. The assignment was based on: (a) the presence of raffinose peak at 8.35 and (b) a stable baseline which would indicate that glucose syrup is not present.

Molasses Analysis

Six samples of molasses (three of beet origin and three of cane origin) were analyzed to determine the level of raffinose present using the IC method. Significant amounts (80-90mg/100g molasses) of raffinose have been reported to be present in cane molasses (ICUMSA, 1982). A test chromatogram showing retention times of raffinose, various kestoses, and nystose is illustrated in Fig. 8. Since it is possible to separate raffinose from galactinol (retention time 1.72) and other oligosaccharides, the determination of raffinose by means of the IC technique should give more accurate result than: (a) the enzymatic method which gives the sum of raffinose, galactinol, polygalactans and free galactose, and (b) the HPLC amino-bonded phase method, because of the superior resolution obtained by the IC method. Results obtained in this study are given in Table V.

Table V

Determination of raffinose in beet molasses
(Results in mg/100g molasses)

Beet Molasses 1	651
2	1685
3	2813

Comparison of the two chromatograms obtained for beet molasses (Fig. 9a) and cane molasses (Fig. 9b) indicated that the peak at 6.48 in Fig. 9b may not be the same as the peak at 6.16 (raffinose) in Fig. 9a. When 10 ppm raffinose elutes at 6.18 min and the original small peak (at 6.48 in Fig. 9b) appears as a shoulder at the tail end of the raffinose peak (Fig. 9c). If this peak at 6.48 in Fig. 9b is indeed raffinose, it would amount to 170mg/100g molasses. At the present time, it is not possible to state definitely whether raffinose is present in the cane molasses. These are several other trisaccharides and oligosaccharides present which must be identified before this decision can be made.

In conclusion, a fast IC method has been developed to differentiate between white beet and cane sugars. Beet sugar products as manufactured are essentially identical with refined cane sugar products except for the presence of raffinose.

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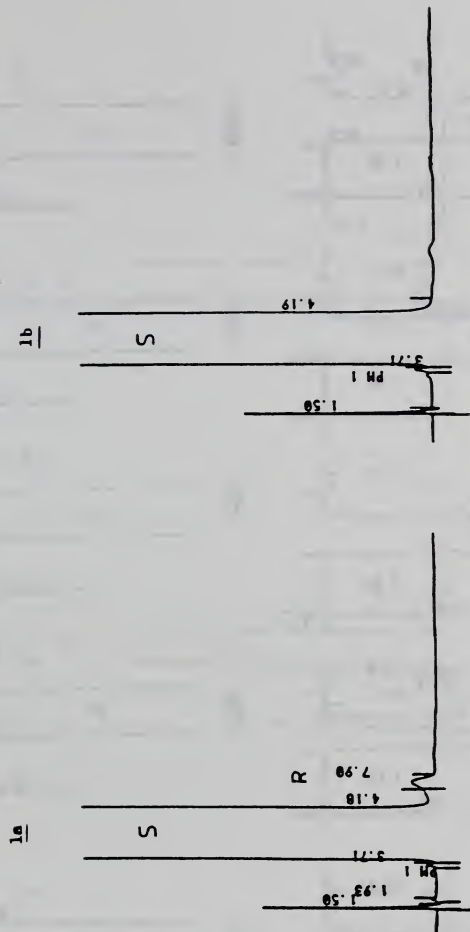


Figure 1. Isocratic analysis of (a) white beet sugar (18,000 mg/l) and (b) white cane sugar (18,000 mg/l). Peaks: S = sucrose and R = raffinose.

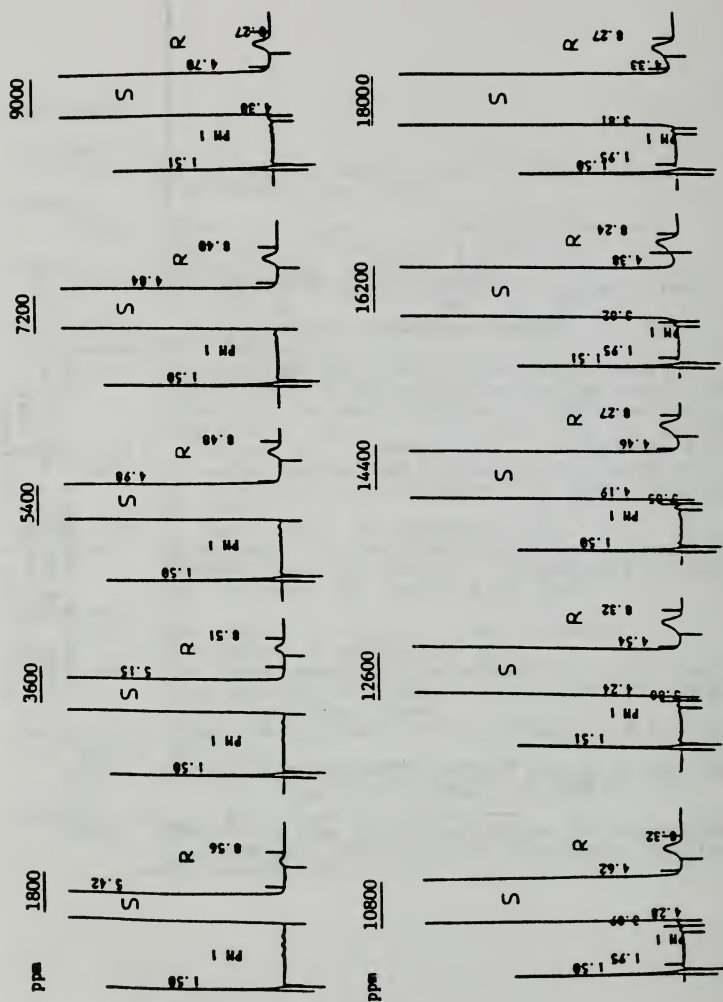


Figure 2. Comparison of the elution profiles of a beet sugar at various concentration (mg/l). Peaks: S= sucrose and R= raffinose.

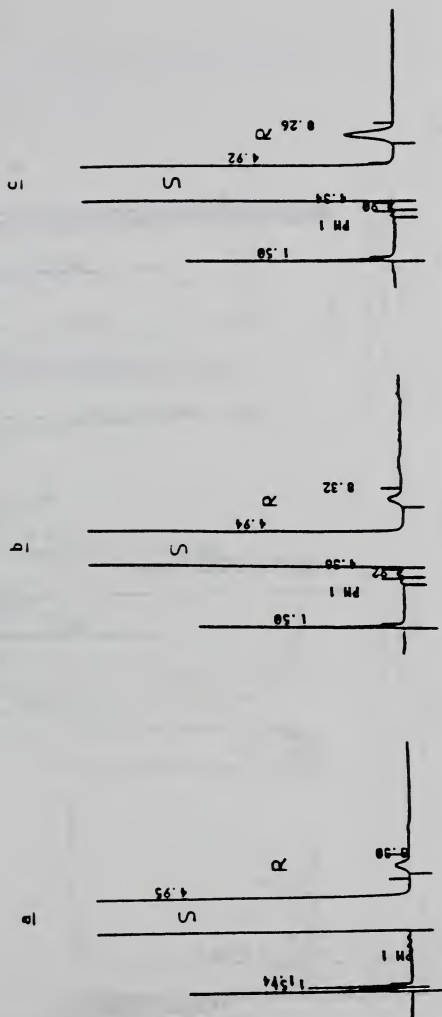


Figure 3. Isocratic analysis of (a) 5000 mg/l white cane sugar + 4 mg/l raffinose, (b) 5000 mg/l white beet sugar, and (c) 5000 mg/l white beet sugar and 10 mg/l raffinose. Peaks: S = sucrose and R = raffinose.



Figure 5.

Separation of sugars in a cola drink sweetened by high fructose corn syrup (a) 5% solution and (b) 0.25% solution. Peaks: Glucose (4.06), fructose (4.51), isomaltose (a, 5.33; b, 5.51) and maltose (a, 13.00; b, 13.02)



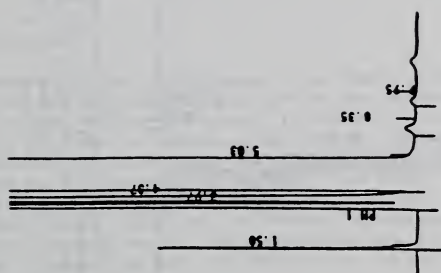


Figure 7.

Separation of sugars in a lime-lemon drink (5%). Peaks: glucose (3.97), fructose (4.37), sucrose (5.03), raffinose (8.35).

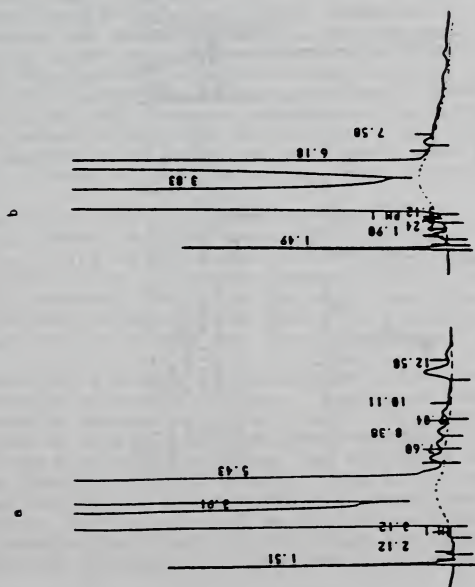


Figure 6. Separation of sugars in (a) lime-lemon drink (5%) and (b) cola drink (5%). Peaks: Invert - glucose + fructose (a, 3.91; b, 3.83), sucrose (a, 5.43; b, 6.18), maltose (a, 12.50).

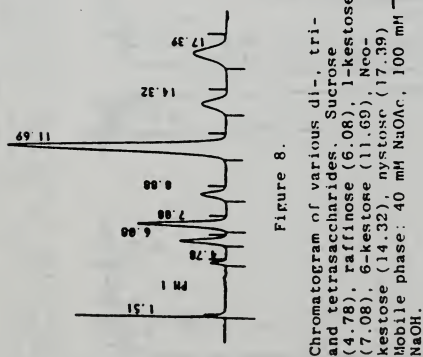
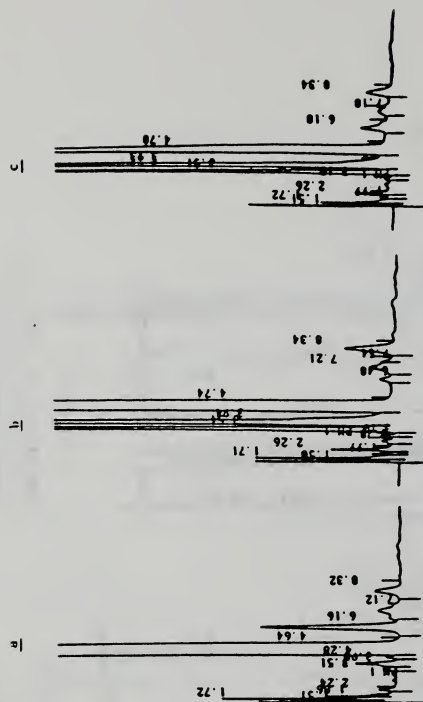


Figure 8.



METHODS FOR THE DETERMINATION OF CARBOHYDRATES BY ION CHROMATOGRAPHY

John C. Thompson

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INTRODUCTION

The determination of sugars in molasses has always been one of the most challenging tasks facing the sugar analyst. Polarimetric methods, while extremely repeatable, are subject to a wide variety of interferences. This makes the results obtained for sucrose little more than an estimation. These techniques also provide no information regarding the other primary constituents of molasses, glucose and fructose. Classically, these two sugars are determined together as "reducing sugars" using methods which, in fact, determine not only glucose and fructose but all substances present which are capable of reducing copper under alkaline conditions.

The introduction of HPLC in the early 70's brought about renewed enthusiasm for molasses analysis. Here at last was a technique that required little or no sample preparation and was capable of determining all three sugars in a single run. Unfortunately, HPLC never reached its full potential within the sugar industry. Much of this lack of acceptance was a result of the precision of many of the analytical methods proposed (ICUMSA, 1982). Some of the blame for this lack of acceptance lies with the sugar analyst who has become accustomed to the very close reproducibility found in pol measurements. However much of it also lies in the non-specific nature of the refractive index detector.

We have found that ion chromatography, or HPIC, offers a significant improvement for the analysis of sugars in refinery products. The selectivity of the separation method combined with the specificity of the detection system makes it possible to use an internal standard technique for sugar analysis. The use of which significantly increases the degree of precision which can be obtained for any method and simplifies sample preparation.

HPIC uses ion exchange mechanisms to effect the separation of compounds as anions or cations. Sugars are separated as anions using sodium hydroxide as the eluent. They are detected by oxidation at a gold electrode where a repeating sequence of three potentials continuously cleans the electrode surface and prevents fouling (Rocklin et al., 1983). This unique combination of ion exchange separation and amperometric detection offers two major advantages, specificity and selectivity. Only those molecules which contain functional groups which are capable of undergoing oxidation at the specific measurement potential applied will be

detected and the mechanism of separation ensures that neutral and basis molecules, even if they are oxidizable, elute in the void volume and do not interfere.

In our laboratory we have developed a method for the simultaneous determination of sucrose, glucose, and fructose in cane refinery molasses using lactose as an internal standard. We have found that the precision of this method exceeds that of other chromatography methods and approaches that offered by polarimetry. As a result we believe that this method offers significant improvements for the determination of these sugars in a wide variety of factory and refinery products of which molasses is perhaps the most challenging.

METHODS AND MATERIALS

All chromatography was performed on a Dionex 4000i IC system equipped with a pulsed amperometric detector and a Dionex 4270 computing integrator. An HPIC-AS6 guard and analytical column were used at ambient temperature controlled between 19 and 20°C. To prevent contact with atmospheric carbon dioxide all eluents were maintained under an atmosphere of helium gas using a Dionex eluent degas module. This reduces the likelihood of carbonate contamination which can effect separation performance.

BDH Aristar-grade sucrose, dried under vacuum and stored over phosphorus pentoxide in a desiccator, was used for the preparation of standard solutions. Glucose/fructose stock solution, containing 0.5g per 100 ml of each monosaccharide, was prepared from sucrose using the method of Eynon and Lane (Schneider, 1979). BDH Analar-grade lactose monohydrate was used on an as is basis as an internal standard in the preparation of both standard and sample solutions. When these sugars were analyzed by HPIC, no peaks additional to those for the specific compounds tested were observed on the chromatograms.

The method, if brief, consists of preparing a standard solution by weighing, accurately, approximately equal amounts of lactose and sucrose and transferring these to an appropriate vessel for dilution. An aliquot of glucose/fructose stock solution is added so that the concentration of the individual monosaccharide is about one tenth that of the sucrose added. For 1 g of sucrose this equates to 20 ml of glucose/fructose stock solution.

A sample solution is prepared in duplicate by weighing, accurately, another portion of lactose and a weight of molasses which contains approximately the same weight in sucrose, so that the ratio of lactose to sucrose in the sample solution is similar to that in the standard. Both standard and sample solutions are diluted stepwise with deionized water until a concentration is reached which is appropriate for injection. Because the ratio of

internal standard to sample is already known this dilution step is not critical and precision volumetric glassware is not required. A 1 ml disposable syringe fitted with a 0.45 μm filter is used for both sample and standard injections. Duplicate injections are recommended.

The order of standard-sample injections and the method of calculation is important to the degree of precision which can be obtained. It has been observed that the detectors response to sugars increases with time, beginning with the time at which the working electrode was last cleaned. This increase, measured in peak area, has been found to be approximately 5 to 15% after 8 hours operation. Furthermore, the response to sucrose increases at a slightly faster rate than the response to lactose so that the calculated relative response factor shows a gradual increase during this period. To counteract this phenomena each sample injection is bracketed by a standard injection. To calculate the appropriate relative response factors to be used for any particular sample injection an average is taken of the before and after standard values. This technique affords the highest degree of precision possible. Other techniques which require less time can be used to give entirely satisfactory results, particularly during short analysis runs or after the initial warmup phase is over; however, all results presented here were calculated using the sample bracketing technique.

RESULTS AND DISCUSSION

Figure (1) shows a typical chromatogram for a molasses sample both with and without lactose addition. Lactose was chosen as an internal standard because it is readily available and, in the quality required, inexpensive. It elutes between fructose and sucrose in a region of the chromatogram which, in our experience, is free from interferences. The order of elution is glucose, fructose, lactose, then sucrose.

Column efficiency for carbohydrates is dependent upon the hydroxide ion concentration of the eluent. In our laboratory we have found that we can increase the selectivity or separation factor between lactose and sucrose from 1.2 to 1.4 by increasing the hydroxide ion concentration from 150 mM to 250 mM. Under these conditions the retention time for sucrose is reduced by 40% allowing for run times of less than 6 minutes.

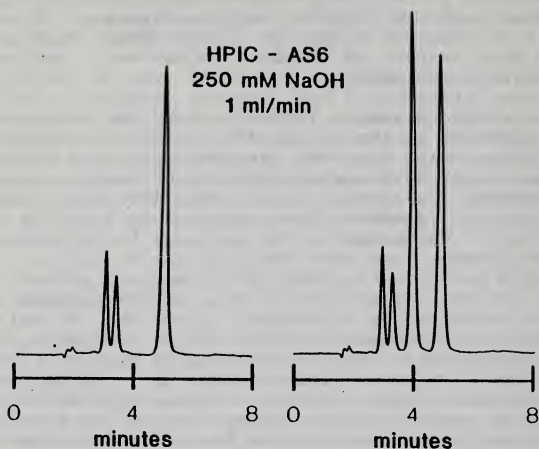


Fig.(1): Typical chromatograms of molasses, with and without lactose addition.

The pulsed amperometric detectors response to all sugars of interest to this method has been found to be linear over the region of concentration encountered. A plot of peak area vs. sugar concentration appears in figure (2). Linear regression provides correlation coefficients exceeding 0.999 for all sugars, indicating excellent linearity. Glucose and fructose exhibit the highest response per unit weight followed by lactose and sucrose. On a molecular level lactose gives the greatest response followed by sucrose, glucose, then fructose.

The method of detection has also been found to be very sensitive. Figure (3) shows a plot of peak area at ever decreasing sucrose concentrations. A 50 μ l loop was used for sample injection and the detector span was set for 300 nA. Such a plot could be used as a calibration curve for the determination of low levels of sugar in materials such as condensate returning to a boiler house. The graph is linear with a correlation coefficient of 0.9987. Given the degree of scatter present it is estimated that levels approaching 100 ppb of sucrose could be determined reliably.

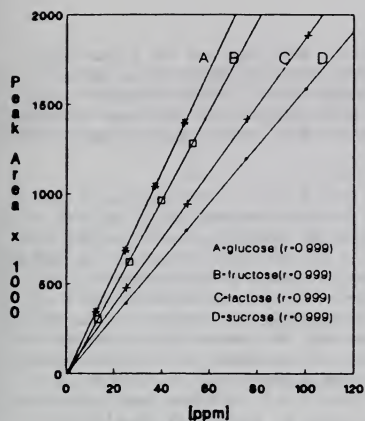


Fig.(2): Detector response.

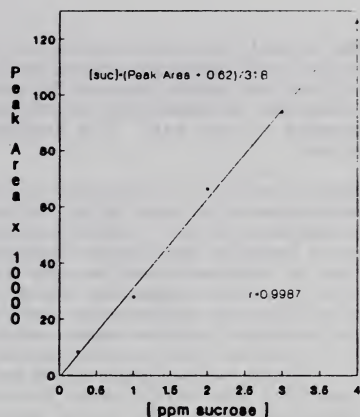


Fig.(3): Limits of detection.

Recovery studies performed using pure glucose, fructose and sucrose in a molasses matrix were performed by spiking solutions containing a known quantity of molasses for which the content of the three sugars of interest had already been established. The method was found to give acceptable recoveries and the results are presented in Table (1). Plots of sugar found vs. sugar added were all found to have slopes which do not differ significantly from unity or intercepts not significantly different than zero, indicating an absence of any constant error or bias (see figures (4) and (5)).

Table (1): Recovery of sugars from molasses

Sucrose			Glucose			Fructose		
added (mg)	found (mg)	recovery (%)	added (mg)	found (mg)	recovery (%)	added (mg)	found (mg)	recovery (mg)
237.9	238.4	100.2	29.3	29.1	99.3	30.9	30.6	99.0
362.5	362.4	99.9	44.7	44.8	100.2	47.1	47.0	99.8
476.8	489.2	100.7	58.8	59.3	100.8	62.0	60.8	98.1
591.6	591.6	100.0	73.0	72.5	99.3	76.9	76.9	100.0
average								
recovery (%)		100.2			99.9			99.2
Std. Dev.		0.32			0.74			0.87

The actual intralaboratory statistical evaluation of precision utilized 15 separate molasses samples derived from cane sugar refineries operating within the Lantic group. All samples contained between 24 and 26% water and ranged in sucrose content between 36 and 48%. All results have been reported on an "as is" basis.

Three levels of interactions which contribute to precision have been examined. The first of these is the variability of the instrument itself. This was examined by evaluating the differences between duplicate injections. The second level of interaction is between the operator and the various preparation techniques such as weighing and diluting. This was established by examining the differences between replicate analysis performed on the same day by the same operator using the same standard solutions and chromatographic conditions. The last level examines the effect of changing laboratory conditions and standards by examining the differences between identical samples analyzed on different days. In total, results from 40 pairs of duplicate injections, 20 pairs of replicate analysis, and 5 pairs of identical samples analyzed on different days were examined.

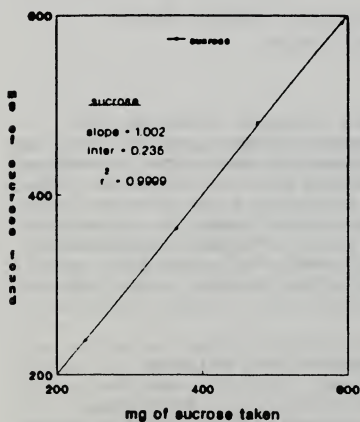


Fig.(4): Recovery of sucrose from molasses

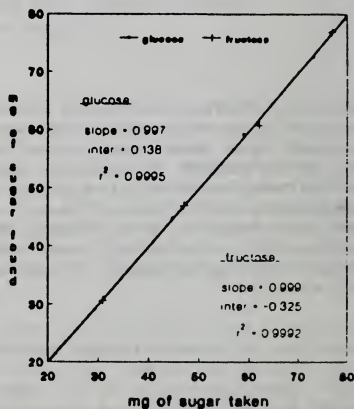


Fig.(5): Recovery of glucose and fructose from molasses

Table (2) shows the results of the between days evaluation of precision. The standard deviation (SD) for a single test for each sugar was determined from the differences between the duplicate pairs using the following formula;

$$SD = \left(\frac{w^2}{2n} \right) \quad (\text{eq.1})$$

where w is the difference between a pair of duplicate results and n is the number of duplicate pairs. Repeatability (r) is defined as the value below which 95% of the differences between duplicates lie (International Standard, ISO 5725). The numerical value of r is determined by multiplying the standard deviation for differences between duplicates by 1.96, this being the number of standard deviations about the mean necessary to fulfil the 95% confidence limit. Since the standard deviation for the differences is 2 times the standard deviation for a single test the repeatability becomes;

$$r = 1.96 \cdot 2(SD) = 2.271(SD) \quad (\text{eq.2})$$

The repeatability of this method over different days performed in the same laboratory by the same operator was found to be 0.14g/100g of molasses for sucrose, 0.07 for glucose, and 0.19 for fructose.

Between replicate repeatability, determined for 20 duplicate determinations, was found to be 0.20 for sucrose, 0.14 for glucose, and 0.15 for fructose. Between 40 replicate injections the repeatability was found to be 0.24, 0.13, and 0.18 for sucrose, glucose, and fructose respectively. All source data can be found in Appendix II.

Table (2): Comparison of between day average results

Molasses sample	Sucrose(%)		Glucose(%)		Fructose(%)	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	39.76	39.82	3.82	3.78	5.00	4.84
2	45.02	44.90	2.40	2.41	3.68	3.73
3	42.78	42.84	3.00	3.01	4.18	4.21
4	36.71	36.67	6.77	6.71	8.21	8.07
5	37.22	37.28	7.12	7.10	5.56	5.54
w^2	0.0268		0.0058		0.0490	
SD	0.0518		0.0124		0.0700	
r	0.14		0.07		0.19	

The method has been found to show a high degree of ruggedness, especially with respect to the ratio of internal standard to sample in the sample solution. Table (3) shows the result of measuring the sucrose content in the same molasses sample at various initial sample weights taken for the same weight of lactose. The results show no significant differences in the percentage of sucrose found. The standard deviation of the average of the 4 results is within the standard deviation for a single injection calculated from the differences between the duplicate injections (eq.2). While there are no real differences in the average results there does appear to be a trend toward lower levels of injection precision at lower concentrations of sugar.

Table (3): Effect of sample concentration on sucrose determined.

Weight of Lactose(g)	Weight of Sample(g)	% Sucrose Determined			w ²
		inject 1	inject 2	average	
0.5031	1.4132	41.83	41.89	41.86	0.0036
0.5027	1.1390	42.12	42.19	42.16	0.0049
0.5051	0.8659	41.90	41.79	41.84	0.0121
0.4978	0.5684	41.83	42.06	41.94	0.0529

average = 41.95					
S.D. = 0.1269				0.1355	

Finally, the only remaining aspect to be examined is the comparison of the results of this method to those of other techniques for the estimation of sucrose in molasses. At Lantic sugar we have traditionally utilized a single polarization method for molasses, using a half normal weight of sample clarified with sufficient wet lead subacetate to provide a suitability clear filtrate for polarization. We have skirted the controversy of true vs. apparent sucrose by referring to the result of this determination simply as the "pol". Figure 6 is a plot of pol vs. HPIC results for sucrose for a variety of molasses samples. The correlation coefficient of 0.97 indicates that there is a close relationship between HPIC and classical sucrose measurements. However, the fact that the slope does not equal unity and the intercept is not zero indicates definite differences between the two methods. The limited data suggests that the differences between the two methods increases as the sucrose content in the sample decreases. This is typical of the error encountered in determining sucrose by single pol techniques as they are influenced by the reducing sugar concentration of the sample and the colour of the lead filtrate, both of which increase as the purity of the sample decreases.

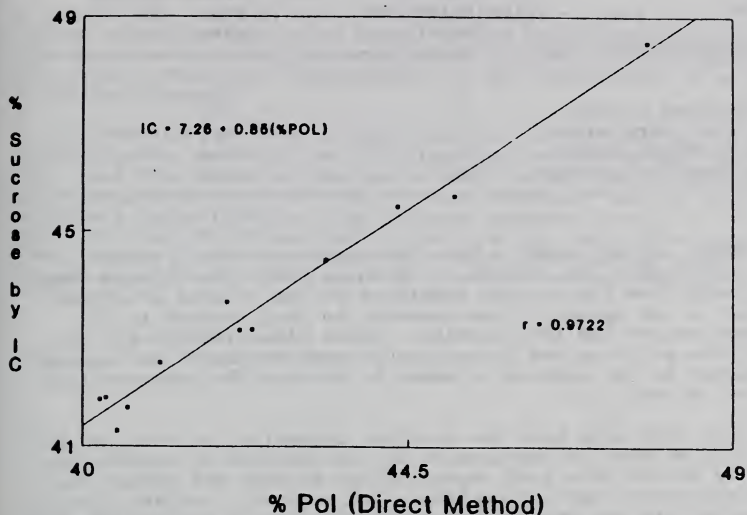


Fig.(6): Comparison to single pol method

SUMMARY

Tables (4) and (5) compare data obtained for the HPIC method with data from some other chromatographic methods reported in the referees report on Subject 8, Sucrose in Factory and Refinery Products, for the 1986 session of ICUMSA.

Table (4): Comparison of HPIC with other chromatography methods.

Method	Repeatability (r)	Range of r Reported
HPIC	0.14	-
GLC	0.5	0.3 - 0.7
HPLC (reverse phase)	0.45	0.3 - 0.6
HPLC (cation exchange)	0.7	0.5 - 0.9

HPIC was found to give better repeatability than any of the methods previously reported.

Table (5): Comparison of run times for various methods.

Method	Average Run Time/Injection (mins)	Total Chromatography Time (mins)
HPIC	6	54
GLC (packed column)	6	126
GLC (capillary column)	3	63
HPLC (reverse phase)	10	240
HPLC (cation exchange)	15	360

The total chromatographic run time required to analyze one molasses sample for sucrose is given in Table (5). These times are taken from the methods submitted to the referee of ICUMSA Subject 8 and appear in the appendix to that subject in the proceedings of the 1986 session. These times include all the replicate weighing and injections of both standards and samples suggested by the authors in order to achieve the repeatability listed in Table (4).

Not only does HPIC have the shortest injection run times of all the HPLC methods it requires fewer replications to achieve the same or better levels of repeatability so that the entire run time approaches that of capillary GLC methods. In fact, day/day repeatability for this method calculated from a single injection of a single weight of sample (total chromatography run time of 18 minutes) is still only 0.3g of sucrose/100g of molasses.

Lantic Sugar has been using ion chromatography in its Saint John laboratory since May of 1988. Besides performing molasses analysis we also use ion chromatography for a variety of routine determinations such as reducing sugars in raw, intermediates, and finished products, including white granulated sugars. We have been able to accurately determine glucose and fructose in quantities as low as 0.05mg/100g of sucrose.

We also perform inorganic anion determinations using suppressed conductivity detection and have found this a useful tool as well for both process control and finished product evaluation.

The principal use of HPIC has been as an adjunct to the analytical services already offered, to both corporation and customers through our technical services laboratory. The function of this facility is to provide accurate analytical data for the purposes of problem solving, raw material and finished product evaluations and new product development. In this capacity we have used HPIC

to isolate the mechanism involved in a specific deterioration problem encountered in brown sugar, solved hygroscopicity problems encountered during the development of fruit chews prepared from natural materials, and assisted in the formulation of a new carbonated beverage.

In our laboratory we have assayed materials as diverse as caramel coated popcorn, uncooked tart fillings and peanut butter. We have found HPIC to be an invaluable tool for the determination of sugars in products and substances where classical methods are completely inapplicable. This includes samples too dark or portions too small to be analyzed by polarimetry, as well as samples which contain a high percentage of sugars from sources other than sucrose which would interfere with classical methods of analysis.

APPENDIX I

The Determination of Sucrose, Glucose, and Fructose in Cane Refinery Molasses by HPIC

1. Equipment

- a. Ion chromatograph, Dionex 2000i or equivalent.
- b. Pulsed amperometric detector.
- c. Electronic integrator, Spectra Physics 4270 or equivalent.

2. Materials

- a. Sucrose, BDH, Aristar, dried under vacuum over phosphorus pentoxide and stored in a desiccator.
- b. Lactose monohydrated, BDH, Analar.
- c. Glucose/Fructose solution, prepared from 2a. using the inversion method of Eynon and Lane (see Schneider, F., Sugar Analysis: ICUMSA methods, (1979) p. 162).

3. Chromatography

- a. column: Dionex HPIC - AS6
- b. solvent: 0.25 N NaOH, prepared from 50% NaOH
- c. flowrate: 1.0 ml/min
- d. column temperature: ambient
- e. sample injection: 10 cm loop, approximate volume 7.5 μ l
- f. detector: Pulsed amperometric, measurement voltage 0.1 V, span 0 - 1000 nA
- g. integration: electronic, by peak area

4. Analysis

a. Preparation of Standard Solution

Weigh accurately 1g of lactose monohydrate and transfer to a 2 l volumetric flask. Weigh 1g of sucrose and transfer to the same flask. Add 20 ml of glucose/fructose solution. Dilute to 2 l with deionized water. Transfer 5 ml into a 100 ml disposable beaker and add 45 ml of di water. Swirl gently to mix.

b. Preparation of Sample Solution

Fill a 1 ml disposable syringe with the sample to be tested. Weigh the filled syringe and then transfer the contents to a 1 l flask. Reweigh the syringe to determine the quantity of sample taken. Weigh 0.5 g of lactose monohydrate and transfer to the same flask. Dissolve the contents with di water then dilute to 1 l. Transfer 5ml to a 100 ml disposable beaker and add 45 ml of di water.

Duplicate subsamples should be prepared for each molasses sample.

c. Standard/Sample Injection

Standards and samples are injected in the following pattern until each molasses subsample has been injected in duplicate;

std, sample (subsample 1), std, sample (subsample 2), std

All standard and sample solutions are injected with the aid of a 1 ml disposable syringe fitted with a 0.45 μ m syringe filter.

d. Calculations

i) Relative response factors (Ks)

$$K_s = \frac{\text{Areas } S}{\text{Areas IS}} \times \frac{\text{Mass IS}}{\text{Mass } S} \quad (\text{eq.1.})$$

where S refers to the sugar (either sucrose, glucose, or fructose) and IS is the internal standard, lactose monohydrate. This relative response is calculated for each sugar for each standard injection.

ii) % Sugar in Molasses (%S)

$$\%S = \frac{\text{Areas } S}{\text{Areas IS}} \times \frac{\text{Mass IS}}{(K_{s1} + K_{st})} \times \frac{100 \times 2}{\text{Mass Molasses}} \quad (\text{eq.2.})$$

where S refers to the sugar (either sucrose, glucose, or fructose) and IS is the internal standard. K_{s1} and K_{st} refer to the relative response factor for the sugar of interest in the leading (K_{s1}) and trailing (K_{st}) standard injection.

iii) Precision

The absolute difference between the %S calculated between duplicate injections should not be greater than 0.25 g per 100 g of molasses. Duplicate injections with differences greater than this should be injected again.

APPENDIX II

Results of Individual Molasses Analysis

SAMPLE	DAY	REPLICATE	DUCROSE		GLUCOSE		FRUCTOSE	
			INJECTION	INJECTION	INJECTION	INJECTION	INJECTION	INJECTION
			1	2	1	2	1	2
A	1	a	39.72	39.81	3.79	3.83	4.97	5.07
A	1	b	39.71	39.79	3.85	3.80	4.97	4.99
A	2	a	39.82	39.93	3.78	3.80	4.82	4.90
A	2	b	39.86	39.66	3.77	3.76	4.79	4.84
B	1	a	45.14	44.94	2.42	2.38	3.69	3.69
B	1	b	44.95	45.04	2.39	2.40	3.64	3.69
B	2	a	44.90	44.79	2.38	2.40	3.68	3.74
B	2	b	44.98	44.92	2.41	2.43	3.72	3.76
C	1	a	42.72	42.84	3.92	2.99	4.20	4.22
C	1	b	42.69	42.86	3.80	2.98	4.15	4.13
C	2	a	42.87	42.86	3.82	3.02	4.20	4.27
C	2	b	42.88	42.79	3.84	2.97	4.22	4.15
D	1	a	36.64	36.79	6.72	6.83	8.13	8.40
D	1	b	36.63	36.79	6.75	6.77	8.21	8.11
D	2	a	36.73	36.61	6.72	6.63	8.02	8.08
D	2	b	36.67	36.67	6.73	6.74	8.01	8.17
E	1	a	37.25	37.18	7.09	7.14	5.57	5.58
E	1	b	37.22	37.23	7.11	7.12	5.56	5.52
E	2	a	37.25	37.23	7.08	7.12	5.47	5.53
E	2	b	37.25	37.37	7.11	7.09	5.61	5.54
F	1	a	42.48	42.58	4.25	4.17	4.96	4.86
F	1	b	42.53	42.60	4.15	4.17	4.81	4.83
G	1	a	41.04	41.09	6.16	6.07	4.70	4.48
G	1	b	41.19	41.02	5.91	6.01	4.41	4.41
H	1	a	41.39	41.35	6.22	6.02	4.98	4.80
H	1	b	41.22	41.17	5.96	5.95	4.81	4.81
I	1	a	43.63	43.71	5.29	5.21	4.56	4.38
I	1	b	43.65	43.71	5.22	5.38	4.37	4.53
J	1	a	43.14	43.12	5.04	5.07	4.88	4.90
J	1	b	43.17	43.26	5.21	5.21	5.05	5.05
K	1	a	44.01	44.35	7.46	7.52	4.71	4.72
K	1	b	44.11	43.82	7.51	7.48	4.71	4.65
L	1	a	43.02	43.10	4.50	4.50	4.95	4.92
L	1	b	43.23	43.30	4.48	4.51	4.96	4.97
M	1	a	44.35	44.44	4.80	3.98	4.35	4.41
M	1	b	44.51	44.52	4.80	4.15	4.37	4.48
M	1	a	45.58	45.82	5.30	5.31	4.68	4.69
M	1	b	45.60	45.60	5.29	5.27	4.73	4.69
O	1	a	48.38	48.48	4.87	4.85	3.93	3.91
O	1	b	48.57	48.62	4.88	4.89	3.98	3.96

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DISCUSSION

Question: It is possible to analyze pentose sugars such as xylose and arabinose simultaneously with sucrose and hexoses.

Thompson: Yes, it is. Xylose appears very quickly in the chromatogram and so does arabinose. They elute before glucose. Sorbitol can also be determined simultaneously in this analysis. The wood sugars require some change in eluent, but can also be determined by this system.

Chairman: Sometimes the conditions must be adjusted. After first using a very dilute sodium hydroxide to separate the sugars, a stronger sodium hydroxide solution can be added post-column for the detection.

Question: With regard to the comparison shown in Figure 6 (Comparison to single pol method), does this apply to beet molasses or cane molasses?

Thompson: To cane molasses in this case. - we are strictly a cane sugar refinery, and show only cane molasses samples.

ION CHROMATOGRAPHY: A COMPARISON BETWEEN ANION AND CATION EXCHANGE HPLC FOR CARBOHYDRATES

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INTRODUCTION

HPLC has been used increasingly over the past 10 years for the analysis of carbohydrates in food and natural products. Although reverse-phase columns (Franco *et al.*, 1987, Ivin *et al.*, 1986, Verzele *et al.*, 1987) and amine-treated silica columns (Johnson *et al.*, 1987, Sayama *et al.*, 1987, Verzele *et al.*, 1987) have been used, most investigators currently prefer cation exchange (CE) columns. These columns do not use expensive or toxic solvents and normally provide adequate separation of most of the common carbohydrates. CE columns, coupled with refractive index detectors (RI), have been used in ICUMSA studies to analyse sucrose in beet and cane molasses samples (Anon 1986, Chorn 1984a) and for the determination of sucrose (S), glucose (G) and fructose (F) in cane molasses (Anon 1990) and other products (Tsang 1988). A review of CE-HPLC has recently been presented by Clarke (1985).

Despite the popularity of CE columns, they do have a number of drawbacks:

- (a) Due to the size exclusion/ligand exchange mechanism, separation power is limited. This together with the non-specificity of the RI detector, can often result in over-estimation of carbohydrates (Anon 1990).
- (b) Although CE columns are useful for the separation of oligosaccharide homologues, they exhibit little ability for separating individual oligosaccharides from one another. Two separate columns are normally needed for mono- and oligosaccharides.

Lee (1989) described the development of anion exchange HPLC columns and the use of pulsed amperometric detection (AE/PAD) as one of the great breakthroughs in carbohydrate analysis in recent years. AE/PAD or ion chromatography (HPIC) has been reported to have a number of advantages over CE/RI:

- (a) AE columns can produce impressive separations in the isocratic mode by merely changing the strength of the mobile phase (Pollman 1989). Although temperature has a small effect on CE/RI resolution little can be done to improve or alter CE separations.

- (b) AE columns can separate structural isomers of oligosaccharides (Anon 1989, Hardy *et al.*, 1988, Thielecke *et al.*, 1989, Tsang *et al.*, 1989). CE columns can only separate oligosaccharide homologues.
- (c) Due to the fact that carbohydrates are weak acids at moderate to high pH, AE columns are more carbohydrate specific than CE columns. In addition, PAD is relatively selective to sugars and alcohols (Anon 1989).
- (d) The PAD is more sensitive than the RI Detector. A sensitivity of 0.1 ppm for glucose has been reported by Welch (1988). Sensitivity of modern RI-detectors is normally about 1 to 2 ppm (Tsang 1988).
- (e) Unlike RI, PAD is relatively insensitive to temperature fluctuations, a 1°C change in temperature has no significant effect on detector drift (Thielecke 1989).
- (f) Gradient separations are possible on AE columns. This is important if mono-, di- and trisaccharides must be separated from one another (Anon 1989). Gradients are not possible when using RI-detectors.
- (g) No column heater is required for AE columns.
- (h) AE columns offer greater mechanical strength than CE columns which means that AE columns can operate at higher flow rates and at greater back-pressures. Rigidity could also result in longer lifetime expectancy.

Research at the SMRI is currently underway to identify the oligosaccharides in cane product responsible for elongating the sucrose crystal. AE/PAD appeared to have considerable promise for assisting in the crystal deformation project and a system was installed in 1989.

CE columns are currently being used at the Institute to determine carbohydrates in sugar products for various routine and research projects.

This paper reviews the recent progress of HPLC/HPIC techniques at the Sugar Milling Research Institute.

EXPERIMENTAL

CE/RI conditions are reported elsewhere (Day-Lewis *et al.*, 1990). AE/PAD conditions were similar to those recommended by Dionex (1989). Important differences are included in the figure legends.

RESULTS AND DISCUSSION

Oligosaccharides and Crystal Elongation

The shape of a crystal grown in the presence of impurities is largely controlled by the faces most retarded by adsorption of the impurities. Sugar cane crystals grown in low purity mass-cuites generally show retardation in the b-direction. Morel du Boil (1985) demonstrated that oligosaccharides were mainly responsible for deformed crystals found in local refinery mass-cuites. The oligosaccharides were isolated using alcohol precipitation followed by separation on carbon-celite columns. Bruijn and Morel du Boil (1986) showed that the oligosaccharide most likely to cause c-axis elongation was 6-glucosyl-sucrose (theand-erose). Thin-layer chromatography was used initially to monitor isolation and fractionation. However the procedure is tedious, time-consuming and subjective. Reverse-phase HPLC, using C-18 columns, initially produced excellent oligosaccharide separations (Morel du Boil 1988), but column life was limited with rapid loss of resolution between critical trisaccharides.

The use of ion chromatography was therefore investigated as impressive separations of oligosaccharides had been reported (Anon 1989). As attempts at obtaining theand-erose from refinery molasses were not successful due to the complexity of the oligosaccharide components, theand-erose was synthesised from sucrose, dextran and dextranase (Miki *et al.*, 1989). The reaction mixture also contained invert, sucrose, the three kestoses, and the iso-maltose series of oligomers. An AE/PAD chromatography of the crude mix is shown in figure 1. Despite the very high concentration of sucrose, theand-erose was well separated from the kestoses and iso-maltotriose.

Theand-erose was purified by removing most of the sucrose, 6-kestose and iso-maltose on a large carbon-celite column. This was followed by a cleanup on Fractogel HW-40(S) where the remaining sucrose, most of the iso-maltotriose and some of the 6-kestose and neo-kestose were removed. An AE/PAD chromatogram of the final fraction indicates that theand-erose is relatively pure with some 1-kestose contamination (fig. 2). The use of AE/PAD for optimising reaction conditions and developing the isolation procedure has been invaluable. The fractionation procedure is currently being used to obtain sufficient stocks of theand-erose for crystal growing experiments.

By comparison, the use of CE/RI is less informative. Although a trisaccharide peak can be seen during theand-erose synthesis, no distinction can be made between theand-erose, the three kestoses and iso-maltotriose (figure 3).

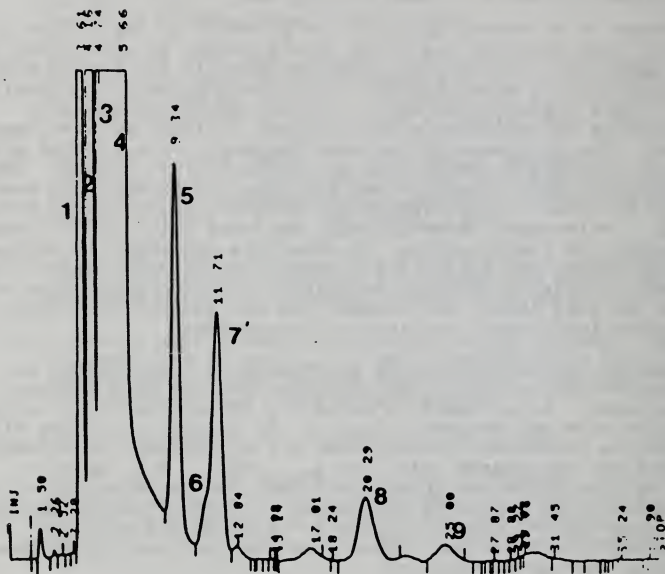


Figure 1.--Sugars in crude reaction mixture, separation by AE/PAD. Mobile phase 100 mM NaOH and 10 mM sodium acetate, flow rate 1 ml/min on CarboPac PA1 and guard column. 1=glucose, 2=fructose, 3=iso-maltose, 4=sucrose, 5=iso-maltotriose, 6=1-kestose, 7=theanderose, 8=6-kestose, 9=neo-kestose.

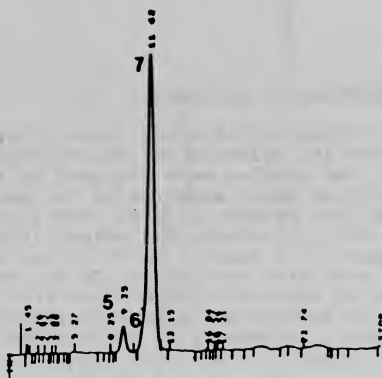


Figure 2.--AE/PAD chromatogram of theanderoses after cleanup of reaction mixture, see legend of figure 1 for details.

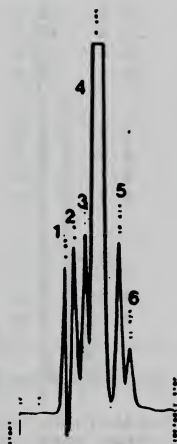


Figure 3.--CE/RI chromatogram of crude reaction mixture, mobile phase was 0.01M Na_2SO_4 , flow rate 0.6ml/min on a Shodex S801S column at 65°C. 1=higher sugars, 2=tetra-, 3=tri-, 4=di-, 5=glucose, 6=fructose.

Glucose, Fructose and Sucrose in Molasses

During a recent ICUMSA collaborative study (Anon 1990), CE/RI was used to determine sucrose (S), glucose (G) and fructose (F) in 6 cane molasses samples. The samples were analysed by 14 laboratories. This was therefore an ideal opportunity to compare AE/PAD with CE/RI. For AE/PAD, the samples were diluted (1:2000) and chromatographed on the Dionex CarboPac PA1 column (figure 4). Separation of the 3 sugars on a typical CE/RI column is shown in figure 5. The samples were also analysed by GC on fused silica capillary columns. The GC results were obtained from 3 independent laboratories and are considered an excellent estimate of the true values for each sugar. A summary of the results can be found in table 1.

Table 1.--Results of CE/RI, AE/PAD and GC procedures for the analysis of ICUMSA Molasses Samples

	SAMPLES						MEAN
	A	B	C	D	E	F	
<u>SUCROSE</u>							
GC	30.5	29.4	30.1	32.3	35.4	33.4	31.9
AE/PAD	30.1	27.7	29.8	31.2	35.6	33.2	31.3
CE/RI-3LABS	30.8	29.6	30.4	32.3	35.5	33.2	32.0
CE/RI-9LABS	30.7	29.7	30.4	32.6	35.1	32.5	31.8
<u>GLUCOSE</u>							
GC	3.6	3.7	4.7	2.5	3.2	3.6	3.6
AE/PAD	3.7	3.7	4.8	2.5	3.2	3.6	3.6
CE/RI-3LABS	3.6	3.7	4.7	2.6	3.2	3.5	3.6
CE/RI-9LABS	3.6	3.7	4.7	3.2	3.3	3.0	3.6
<u>FRUCTOSE</u>							
GC	6.1	7.2	7.8	5.5	5.1	4.3	6.0
AE/PAD	6.1	6.9	7.6	5.3	5.0	4.3	5.9
CE/RI-3LABS	6.2	7.3	7.9	5.5	5.2	4.3	6.1
CE/RI-9LABS	6.3	7.4	8.0	5.0	5.2	5.1	6.2

The main findings of the interlaboratory study were that:

- (a) Three of the laboratories, using the CE/RI procedure, produced results that were extremely close to the GC-data, the lab-to-lab reproducibility for each sugar was also most acceptable ($R_s=0.6$, $R_Q=0.6$ and $R_F=0.7$).



Figure 4.--AE/PAD chromatogram of sugars in an ICUMSA cane molasses sample, mobile phase 100mM NaOH. 1=glucose, 2=fructose and 3=sucrose.

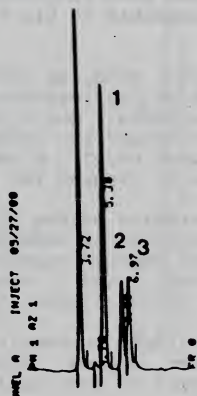


Figure 5.--CE/RI chromatogram of sugars in an ICUMSA cane molasses sample, mobile phase 0.01M Na₂SO₄ on Shodex S801S column at 65°C. 1=sucrose, 2=glucose, 3=fructose.

- (b) The CE/RI results for all 9 labs produced results that were somewhat higher than the GC results indicating co-elution of impurities for certain labs. However, more serious than the bias was the high scatter experienced between laboratories. The interlab reproducibilities (R) were 1.6, 0.9 and 0.8 for sucrose, glucose and fructose respectively. All these are on the high side.
- (c) The repeatability (r) results from sucrose ($r=1.4$) with AE/PAD were disappointing. However the precision for the monosaccharides was good ($r_0=0.1$ and $r_f=0.5$).
- (d) The agreement between GC and AE/PAD for the two monosaccharides was excellent. This was not the case for sucrose where the poor repeatability probably affected the mean values for AE/PAD.

These preliminary results are encouraging and to-date both CE/RI and AE/PAD techniques look promising for routine analyses of sucrose, glucose and fructose in sugar cane products. Interesting ideas on hybrid systems for sugar analysis are discussed in the next section.

Hybrid Chromatography

The discussion until now has been restricted to the comparison of AE/PAD and CE/RI. It is possible to mix the components from both systems:

- (a) AE/RI. In a recent study on oligosaccharides, Thielecke (1989) compared the retention times of sugars on anion exchange columns. One system used PAD whilst the other used an RI-detector. AE/RI should produce greater selectivity than CE/RI. A comparison between these two HPLC systems is planned for 1990.
- (b) CE/PAD. If separation on the CE column is adequate and additional selectivity or sensitivity is required then CE/PAD could well improve results. This has been reported by Johnson (1987). Sodium hydroxide is mixed with the column effluent prior to detection.

CE/RI often results in overestimation of sugars, especially in molasses samples. CE/PAD will therefore be compared with CE/RI at the Institute during 1990.

Trace Sugars in Condensates

HPLC has been used by several investigators to determine trace sugars in waste waters or factory condensates (Tannock 1984,

Chorn 1984b, Tsang 1988). These tests have been carried out to determine the accuracy of existing empirical colorimetric tests.

Modern RI-detectors are fitted with large heat-sinks and sophisticated temperature controllers. This has resulted in a significant improvement in sensitivity. Tsang (1988) using the Waters 410 RI could detect trace amounts of sucrose down to about 1 ppm. A superficial investigation of the Erma ERC-7512 RI and the Dionex PAD detectors was carried out at the Sugar Milling Research Institute.

Examples of chromatograms obtained from the two systems are included in figure 6. Although not directly comparable (different injection volumes, flow rates etc.), it is apparent that PAD is more sensitive than RI. However sensitivity is better only by a factor of about 2-5 times. This is very different from the 100 fold claimed by Pollman (1989). Background noise from the PAD-2 was nearly 2nA. A 50 μ l aliquot of a 50 ppm sucrose solution produced 450nA response, inferring a minimum detectable quantity of 0.5 ppm for sucrose.

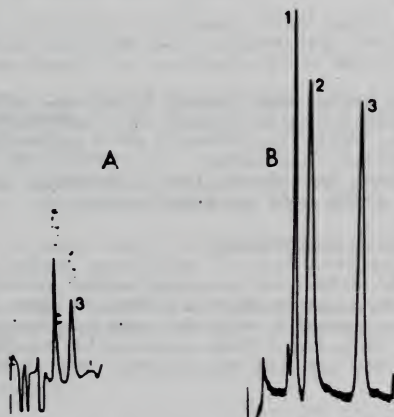


Figure 6.--Sensitivity test for HPLC detectors.

A = CE/RI, 3 = 5 ppm sucrose

B = AE/PAD, 3 = 2.5 ppm sucrose

Both CE/RI and AE/PAD were used to analyse 10 condensates from different raw sugar factories. Although both techniques were capable of determining F, G and S, for convenience only total sugars are compared to the phenol-sulphuric acid results (table 2).

Table 2.--Comparison of phenol/H₂SO₄, CE/RI and AE/PAD for sugar traces (ppm) in condensates

Sample	Phenol/H ₂ SO ₄	CE/RI	AE/PAD
vapour 1	21	18	17
vapour 2	2	2	1
vapour 3	1	1	2
vapour 4	37	26	26
E2	0	0	0
E4	30	28	29
E5	26	26	29
Exhaust 1	0	0	0
Exhaust 2	12	0	1
Feed	3	0	0

Both chromatographic techniques showed excellent agreement and clearly either procedure could be used for condensate analysis. For routine analysis, CE/RI is probably more convenient, as mobile phase preparation is simpler. If carbonates are not completely removed from the eluent for AE systems, decreasing retention times and drift will be experienced.

Monitoring Inversion in Refineries

AE/PAD can be used to determine monosaccharide levels in refineries. Any increase in glucose is an indication that inversion is occurring. AE/PAD was used to monitor possible inversion in a back-end refinery. Melt and return streams were composited weekly and analysed for ash and monosaccharides (Table 3).

Table 3.--Monosaccharides and ash in refinery samples.
(% on sample)

Sample	G	F	Ash	F/G	G/ash	F/Ash
M1	0.06	0.06	0.15	1.00	0.40	0.40
R1	0.35	0.17	1.26	0.49	0.28	0.13
M2	0.04	0.04	0.11	1.00	0.36	0.36
R2	0.35	0.19	1.11	0.54	0.32	0.17
M3	0.07	0.07	0.15	1.00	0.47	0.47
R3	0.45	0.22	1.23	0.49	0.37	0.18
M4	0.05	0.06	0.17	1.20	0.29	0.35
R4	0.52	0.27	1.75	0.52	0.30	0.15

From table 3 it would appear that:

- (a) the F/G ratio decreased significantly between melt and return (1.1 to 0.5) indicating considerably more fructose than glucose destruction during refining.
- (b) the G/ash ratio dropped during refining, which is an indication that excessive inversion probably was not occurring.

Although CE/RI could also be used for troubleshooting inversion in refineries, AE/PAD is preferred because:

- (a) it is more sensitive than CE/RI and baseline stability is better resulting in more precise results.
- (b) the elution order on AE/PAD ensures that low levels of monosaccharides are eluted before sucrose overloads the column.

Other Applications

(a) Analysis of sugars in bread. An incentive scheme has recently been introduced by the S A Sugar Association to encourage bakers to add increasing amounts of sucrose to flour during bread production. Sugar addition is claimed to improve flavour, enhance crust appearance and to act as an additional substrate for yeast production. During dough-making sucrose is rapidly converted to glucose and fructose. Hence fructose levels in dough or bread should be related to the initial sucrose level. AE/PAD was used to monitor loaves of bread produced from flour

containing increasing amounts of sucrose. Maltose, trehalose and sucrose are well separated by AE/PAD. No residual sucrose could be detected confirming that all sucrose had been consumed. A typical chromatogram is shown in figure 7a. The corresponding CE/RI chromatogram is presented in figure 7b. Both AE/PAD and CE/RI were used to determine the fructose contents of loaves prepared from flours with known levels of added sucrose. Excellent agreement was obtained between sucrose added to flour and fructose found in bread (correlation coefficient = 0.99). The procedure is to be used to audit the sugar rebate scheme.

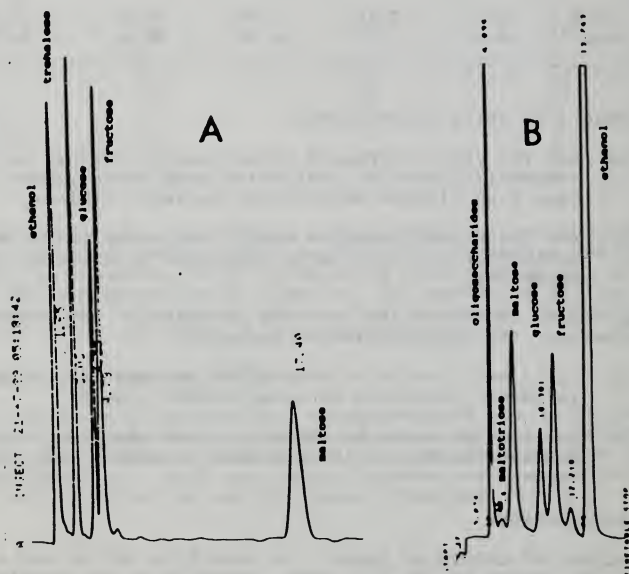


Figure 7.--Sugars in bread using AE/PAD and CE/RI.

A = AE/PAD

B = CE/RI

(b) Fructose block in beer production. Many of the local S A breweries have moved away from using sucrose as an adjunct to wort during beer production. Cason (1987) claimed that glucose is preferentially utilised over fructose by the yeasts during the brewing process. The residual concentrations of fructose can apparently spoil the bitter taste of certain lagers. The established organoleptic profile of these beers can be distorted if fructose remains above its taste threshold of 300 ppm. In order to monitor trace sugars in beers from either glucose or sucrose supplements, both CE/RI and AE/PAD were used to determine residual concentrations of F and G in beers (table 4).

Table 4.--Analysis of sugars in beers by CE/RI and AE/PAD

Beer	Fructose		Glucose	
	CE/RI	AE/PAD	CE/RI	AE/PAD
1	625	420	725	800
2	1350	1350	1350	1325
3	700	625	675	850
4	0	0	50	100
5	0	0	50	125
6	1050	960	0	30
7	0	70	35	40
8	0	130	30	40
9	1050	880	950	925
10	350	260	0	50
Average	500	470	385	425

It is apparent from table 4 that:

- (a) both CE/RI and AE/PAD gave similar results for the monosaccharides. Therefore either procedure could be used for this investigation.
- (b) the level of monosaccharides varied significantly from beer to beer. This was probably due to the nature of the carbohydrate source and the yeast concentration used.
- (c) beers 6 and 10 had high levels of fructose with little or no glucose present.

This project has only just started, CE/RI and AE/PAD should prove invaluable in determining whether high levels of sucrose can be used in beer production without adversely affecting taste.

CONCLUSIONS

Both AE/PAD and CE/RI systems (together with GC) form an indispensable chromatographic service to the local sugar industry. AE/PAD has proved itself to be the method of choice from monitoring and isolating oligosacchrides implicated in crystal deformation. As mobile phase preparation is easier, CE/RI is preferred for automated analysis of sugars in condensates, juices, molasses, fermented liquors and distillery samples.

ACKNOWLEDGMENT

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DISCUSSION

Question: You've shown that anion chromatography is a very good and sensitive method to determine carbohydrates. I believe it would be especially appropriate for determination of oligosaccharides - raffinose, kestoses, etc. - in molasses and factory materials. But do we need this high sensitivity to determine only sucrose and invert sugars in routine analysis?

Also - in the cation exchange system, today we use other columns, for example in the lead form. We have a very good differentiation of various carbohydrates at high sensitivity.

Schaffler: My feeling is that the sensitivity of the PAD detector has been heavily emphasized. Some papers claim that PAD sensitivity is about 100 times that of a normal high sensitivity refractive index detection, for example Waters Associates Model 410, we never found that. If you compare the PAD system with older refractive index detectors without temperature control, that difference might be so. But compared to current R.I. systems, we found the PAD had 2 to 5 times the sensitivity. With low concentrations of sugars, for example, condensates, 1 ppm can be determined quite easily. On your point about cationic columns - you can change columns, going to a 4% cross-linked resin, to improve separation of oligosaccharide, but you won't be able to achieve the selectivity of the IC system. If you are looking at homologues, the cation system is fine. But in cane or beet molasses, with tri- and tetrasaccharides, then in my opinion its necessary to use ion chromatography with PAD detection.

Question: Thanks for a very interesting paper, I noticed in the first paper an interesting use of lactose as an internal standard for IC. Two questions arise: first, in the comparative studies you report of IC with GLC, was an internal standard used. Secondly, would you like to comment on the use of internal standards in IC techniques.

Schaffler: When we were developing ion-exchange methods, we tried a number of internal standards - xylitol and some others. With an automated system, we found no improvement in the precision when an internal standard was used. If you are using a fixed loop system, I don't think its necessary to use an internal standard. We checked one sucrose results by using trehalose as an internal standard, and found no improvement. But looking at Jack Thompson's work, I think we'll try to change some of our conditions.

ANALYSIS OF SUGARS BY HPIC

R. W. Plews

Tate and Lyle plc

INTRODUCTION

Sugar solutions have been analysed by HPIC using a Dionex 4000i system fitted with an "AS 6" anion column, using Pulsed Amperometric Detection (PAD), with gold working electrode.

HPIC analysis of molasses produces lower total sugars than those by the classic Fehlings method. The HPIC method is a direct analysis of the filtered sugar solutions. In the classic technique the total sugars are inverted for analysis, where some higher polysaccharides e.g.: starch, could be changed to reducing sugars which inflate the result.

There is better comparison with GLC results, although the HPIC method still produces lower results, probably due to the GLC samples being derivatised and not pre-filtered.

Problems found with the HPIC method include the poor reproducibility $\pm 2\%$ on peak, possibly due to oxidation or reduction products within the cell causing baseline drift, background noise and changing analytical sensitivity. Glucose and fructose of less than one part per million in the presence of high sucrose concentrations, can not be determined due to the sucrose peak spreading. The higher order polysaccharides show poor detection at low concentrations due to lower sensitivities.

In the experience gained so far analysis by HPIC gives a picture of the sugar qualitatively but not quantitatively to better than $\pm 2\%$. Investigations are continuing to improve the reproducibility to a point where the technique could be used for routine process control and final product analysis.

We have found many problems with the use of HPIC analysis for analysing carbohydrates within the refinery.

First basic problem is the separation of glucose and fructose: they do not resolve using the recommended eluant for the "AS 6" column (0.15 molar sodium hydroxide); this is shown in Fig. 1. Poor resolution can lead to problems with reproducibility and therefore the accuracy of the system. The stability of the calibration is poor, this is possibly due to precipitation within the PAD cell, that of an auric compound i.e.: auric hydroxide $\text{Au}(\text{OH})_3$ or auric oxide Au_2O_3 . The precipitate is formed with only the hydroxide eluant, but seems to increase with the reactions of carbohydrates passed through the cell. It is therefore necessary

to calibrate the instrument prior to each analysis with a standard close to that of the samples to minimise error due to drift.

To try and resolve the problem with separation a range of hydroxyl ion concentrations were used to give eluants of 0.1, 0.15 and 0.2 molar sodium hydroxide. No improvement in the resolution of glucose and fructose although a decrease in retention time was noted with increasing hydroxyl ion concentration. This work was done at a temperature of 20°C, it was therefore decided to buy a temperature controller for the column and to try the effects of temperature on resolution and retention. The retention times were found to decrease with increasing temperature but at temperatures about 40°C peak trailing started. The resolution of glucose and fructose was unchanged as shown in Fig. 2. The most significant change with temperature was that of stability, for it had been noted that at 20°C there was an increase in the analysis of carbohydrates with each sample run. Repeat analysis of the same sample, showed an increase in total sugars by as much as 8% in the six runs, (see Fig. 3). With increasing temperature the upward drift in the analysis has been reduced. We have settled on the temperature of 40°C for increased stability without peak trailing.

We have tried modifying the eluant, by adding sodium acetate at levels of 0.5, 1.0, 5.0 and 10.0 millimolar to 0.15 molar sodium hydroxide, these produced a slight change in retention time but did not improve the separation of glucose and fructose. The addition of magnesium acetate had no effect but that of zinc acetate produced a perfect separation with a concentration of 0.5 millimolar, see Fig. 4. Although the resolution is good, the stability decreased, therefore all work to date has been done with an eluant of 0.15 molar sodium hydroxide at a temperature of 40°C and a flow of 1 ml/minute.

With the eluant of 0.15 molar sodium hydroxide and a flow of 1 ml/minute, the PAD cell was checked for optimisation for carbohydrates. This was carried out using a standard of glucose, fructose and sucrose. Initial values of E1, E2 and E3 were set to E1 0.2v, E2 0.6v and E3 -0.8 volts. Each one of the E values were changed one at a time over a voltage range, the other two values remained set at the initial settings. Results for maximum total sugars were obtained for each E setting, optimum values from the graph shown in Fig. 5. are E1 0.2, E2 0.5 and E3 -0.8.

Having looked at all the above possibilities, it is concluded that the PAD cell and detector are operating within the design limits of $\pm 1\%$. But the analytical column fails to give good resolution of the glucose and fructose, we therefore intend to look at the use of the finer *CarboPac column to resolve this problem. *(Suggested by W.S. Charles Tsang.) Due to the detector sensitivity of $\pm 1\%$ on a calibration it is possible for a subsequent sample to be in error by up to $\pm 2\%$. Which at best would

mean an error on a typical molasses of, at 1%, ± 0.5 Brix on total sugars and at 2%, ± 1.0 Brix.

Due to the sequential system drift, calibration prior to each sample is required. It has been found by multi-calibration the response for carbohydrates up to cellobiose is linear with this column and cell. With the standard eluant, carbohydrates of a greater retention time than cellobiose show lower sensitivity. Because of the lower area to concentration, errors are larger. (See calibration graphs Figs. 6-9)

Comparison between analysis carried out by HPIC and classic Fehlings on products across the refinery show that with the increase of impurities in the product, there is a proportional decrease in the analysis by HPIC. Analysis of mixed pure carbohydrate by both methods produce the same analysis within the system limits, as shown in Fig. 10 and Fig. 11 a,b. It is possible that the analysis done by HPIC although not accurate may be nearer to the truth than that of the classic method. This is due to the HPIC method requiring no pre-treatment other than filtration, the classic methods is more likely to be in error due to impurities such as e.g.: starch and other polysaccharides being hydrolysed during the preparation. This would be consistent with the overall increase in the classic analysis due to the increase in concentration of impurities. Alternately the impurities would have to suppress detection within the PAD cell. The later is not the case, this can be shown by injecting a known standard along with the sample. By injecting lactose with the calibration and then a known amount with the sample it is found that there is no suppression of the lactose. This would indicate that the analysis of all species is correct.

Analysis of invert in refined sugars is not possible due to the very low levels of invert present typically 0.01%, this is equivalent to less than 20ppb. on a standard sample with 150mg/l sucrose. By increasing the sucrose level to that suitable to read the invert, inverts present would be swamped by the spread of the sucrose peak.

Raw sugar analysis can be done, using a larger sample to give a sufficient level of invert to measure. But the sucrose level is then too high to measure and therefore only inverts can be measured with this sample, although the measured invert will still be very low at less than 10ppm on peak, with an initial sample strength of 5000mg/l. Due to the low levels of invert the analysis is more likely to be in error due to base line stability. Analysis of the sucrose can be done on a separate sample, but due to the raw sugar being 99% sucrose, the instrument error of $\pm 1\%$ on peak would make the analysis meaningless. HPIC analysis of all raw sugar is lower than that by the Fehlings method. (Note: using higher sample levels will decrease the column life because of impurities in this sugar.)

Analysis of molasses by HPIC produce lower result than that of the Fehlings method. The sample composition suits the use of HPIC because of the convenient invert: sucrose ratio. A sample strength for analysis in the order of 400mg/litre gives a sucrose peak of 150mg/litre and 30mg/litre each of glucose and fructose Fig. 12 shows a typical molasses chromatogram, results tabled below.

Xylitol	0.10%
Sorbitol	0.07%
Arabinose	0.10%
Glucose	6.86%
Fructose	7.23%
Sucrose	35.87%
Cellobiose	0.48%

<u>HPIC ANALYSIS</u>		<u>FEHLINGS ANALYSIS</u>
Invert	14.67	16.58
Total Invert	52.34	57.57

HPIC For Qualitative Analysis

The HPIC system is a very good instrument for qualitative analysis, as shown by the analysis of Zubes*. Here it shows that by the addition of flavours, the invert composition has been changed by the addition of fructose as a fruit sugar see table below.

*(Zubes; a confectionery product)

Samples of five high boiled sweets were received for analysis.

	Original Zubes	Black Currant Zubes	Cherry Zubes	Strepsils (Boots)
Karl Fischer water %	2.9	3.3	2.9	2.5
Fehlings invert %	14.2	17.9	16.5	21.4
HPIC total invert %	5.3	7.7	6.7	8.9
Glucose %	5.3	6.8	6.2	7.9
Fructose %	<0.1	0.9	0.5	1.0
pH 10% Aq.	9.0	3.5	3.5	3.6

For quantitative analysis the system is not so reliable as shown by the table sample reproducibility.

Sample Reproducibility

A sample of molasses was prepared for analysis by HPIC. Six samples in all were weighed out and made up in volumetric flasks. The weights were noted approximately 300 milligrams.

Results are tabulated below.

<u>Sample Wt.</u>	<u>Glucose</u>	<u>Fructose</u>	<u>Sucrose</u>	<u>Total sugars</u>
mg	%	%	%	%
-	6.16	6.40	34.99	47.55
281.6	6.29	6.43	36.38	49.10
274.0	6.26	6.11	36.05	48.42
288.5	6.34	6.49	35.49	48.32
314.0	6.12	6.35	35.45	47.92
292.9	6.17	6.72	35.94	48.83
average	6.22	6.42	35.72	48.36
mean	6.23±1.76	6.42±4.75	35.69±1.95	48.33±1.60

(eluant 0.15 molar NaOH; Flow 1.0 mls/min.)

Below are results for the same sample analysed by G.L.C.

<u>Glucose</u>	<u>Fructose</u>	<u>Sucrose</u>	<u>Total sugar</u>
6.46	7.11	35.31	48.88

It can be seen that the G.L.C. analysis is higher on the invert sugars.

Other molasses samples, show the same trend.

<u>Method</u>	<u>Glucose</u>	<u>Fructose</u>	<u>Sucrose</u>	<u>Total sugar</u>
HPIC	6.75	6.68	34.04	47.47
G.L.C.	7.00	7.63	34.10	48.73

From the above tables it can be seen that in the main results by HPIC fall within plus or minus two percent although they do not directly compare with G.L.C. analysis or that of Fehlings which produce higher results than both HPIC and G.L.C.

Starch Analysis

The analysis of the starch content of raw sugar by HPIC is a method which we have looked at. We have extracted starch from a sugar solution by the Alexander method. (Proc. South Afr. Sugar Technol. Assoc., 1954, pp. 100-104)

Method

Dissolve 100g sugar in 100cm³ distilled water.

Add 20cm³ N HCL, stir.

Add 300cm³ methylated spirit, stir and leave for 2 hours at room temperature.

Add 2g filter-aid and stir.

Filter through a 7cm filter paper pre-coated with 1g filter-aid under vacuum.

Wash cake with,

a) 100cm³ 75/25 meths/water

b) 50cm³ hot, undiluted meths

The cake is dried by drawing air through for about 5 minutes.

Analysis by HPIC

Take cake, add 50cm³ distilled water and 5cm³ of 6.34 molar HCL.

Heat to 60°C for 20 minutes.

Adjust pH to 7.0

Make to 100cm³ for analysis.

It was found using this method that starch could be hydrolysed to glucose for measurement, but we also found there was a problem with sucrose being still present after the washing. Further work on the separation of starch is needed; this method then might also be applied to total polysaccharides.

Conclusion

The HPIC method of analysis for sugars and syrup within the refinery offers a qualitative analysis rather than quantitative, due to an accuracy of at best $\pm 1\%$.

However it does lead to a possible quantitative analysis of starch and other polysaccharides by reducing them to invert sugars, although this area would require much more work.

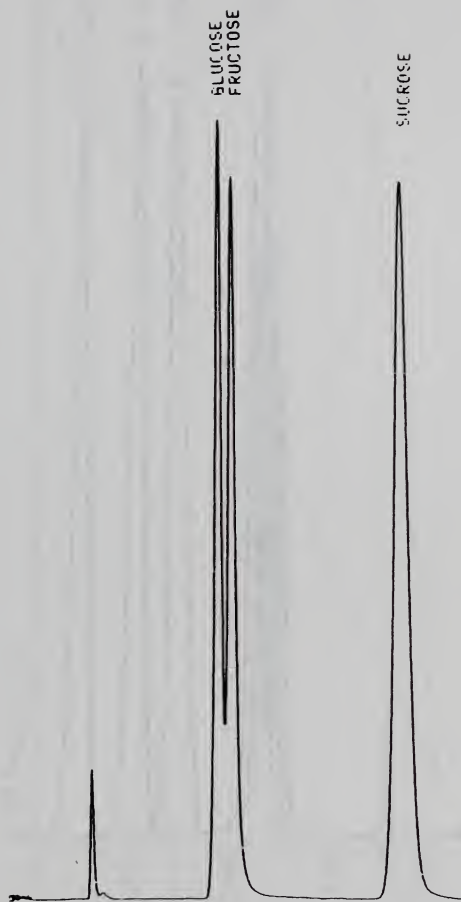


Figure 1. Poor separation of glucose and fructose

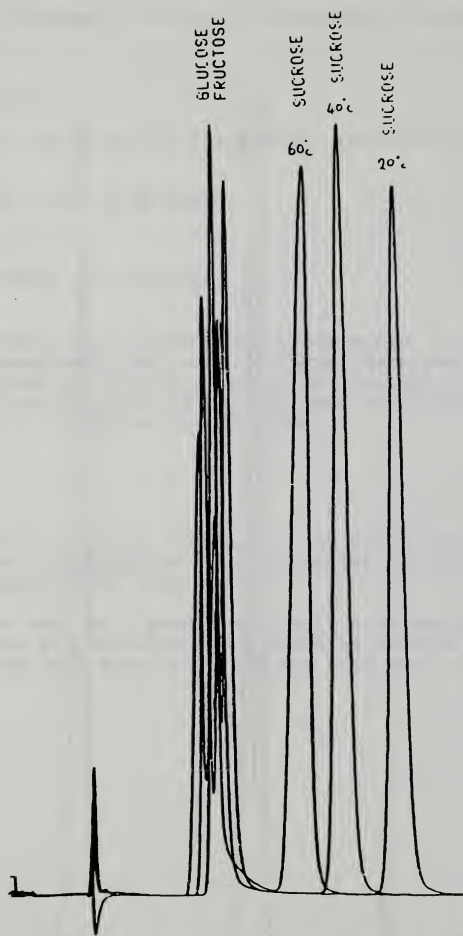


Figure 2. Resolution of glucose and fructose

HPIC SEQUENTIAL SYSTEM DRIFT.

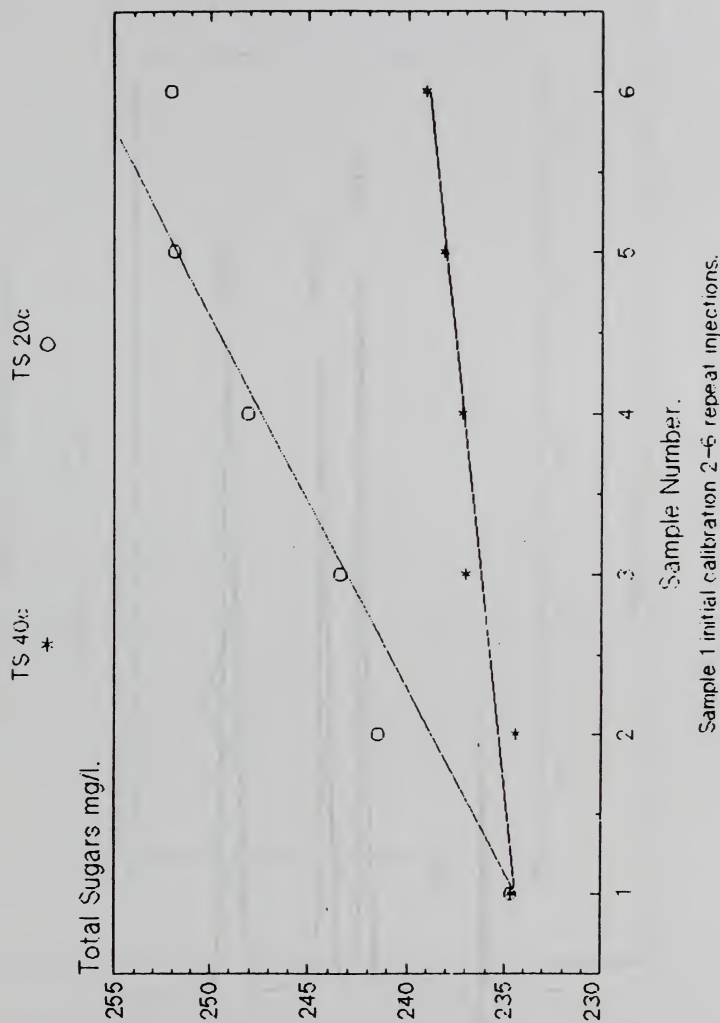


Figure 3. Repeated analysis on HPIC

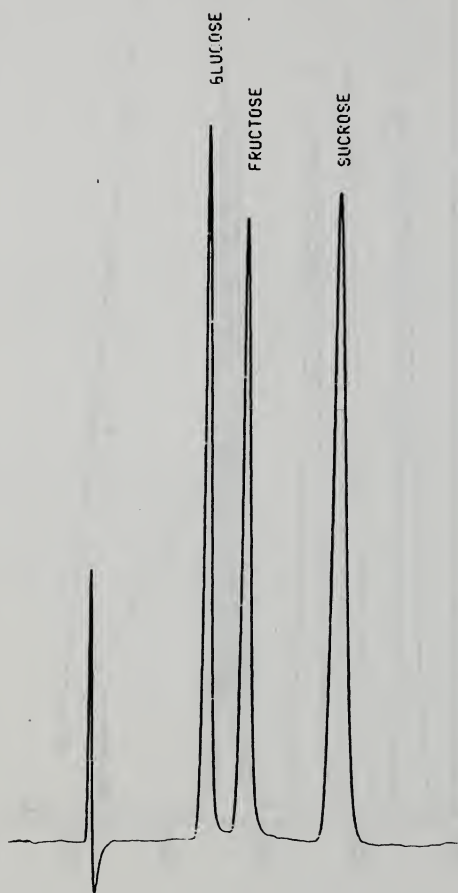
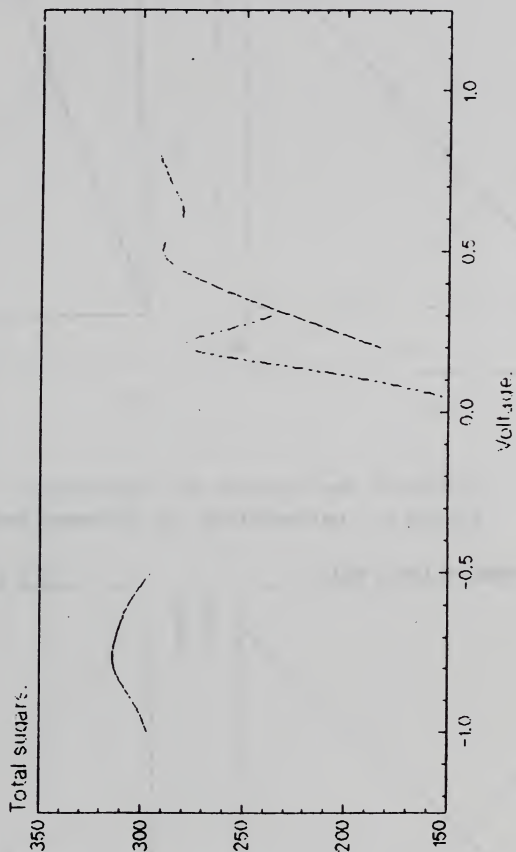


Figure 4. Effect of zinc acetate on separation

E VOLTAGE VALUES.

Flow 1.0mls/m, 0.15MNaOH eluant.

E3 E2 E1



Optimum value E1 0.21, E2 0.52, E3 -0.77

Figure 5. Voltage settings.

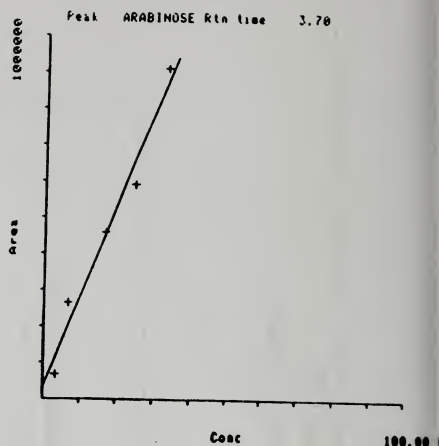
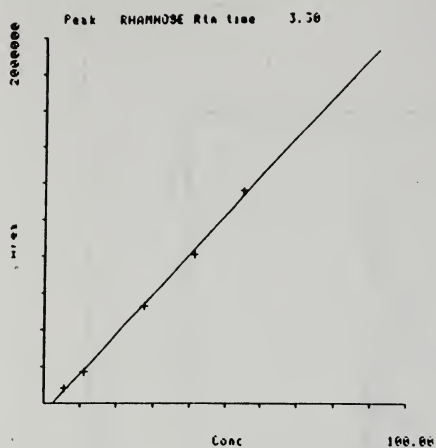


Figure 6. Calibrations for rhamnose and arabinose

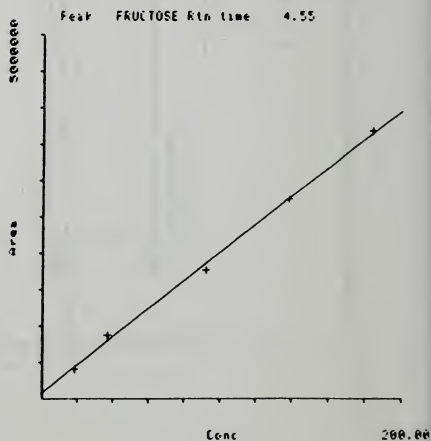
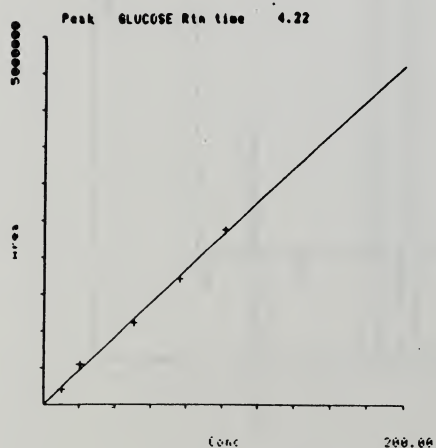


Figure 7. Calibrations for glucose and fructose

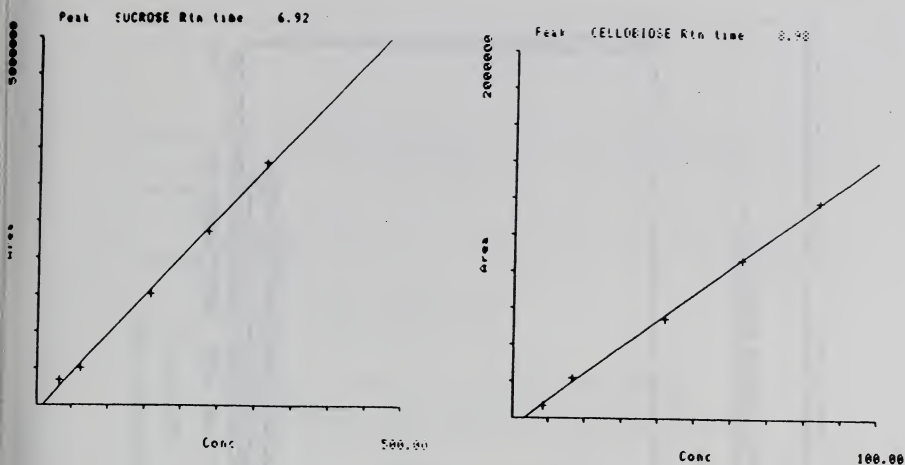


Figure 8. Calibrations for sucrose and cellobiose

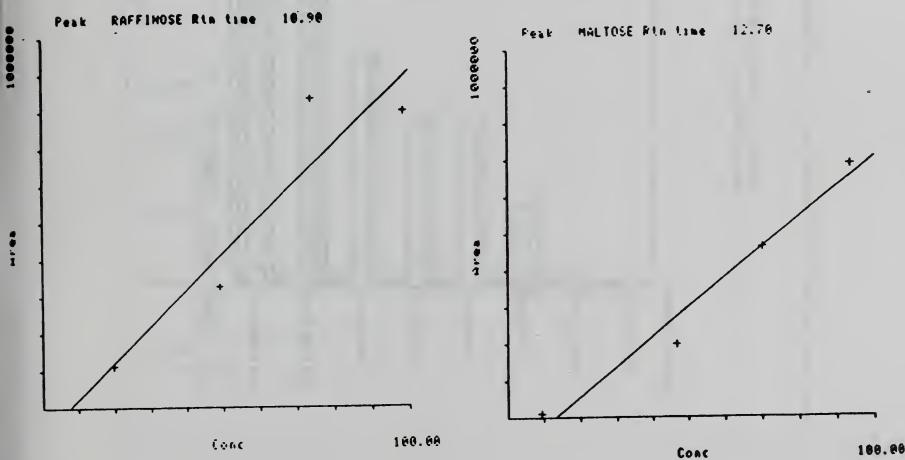


Figure 9. Calibrations for raffinose and maltose

Invert difference between Fehling analysis and HPIC.

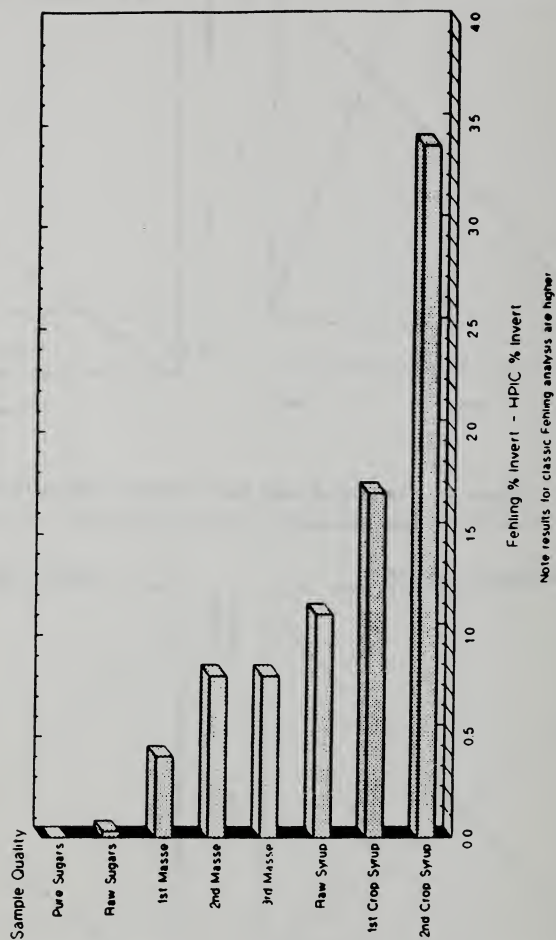


Figure 10. Comparison of Fehlings and HPIC results

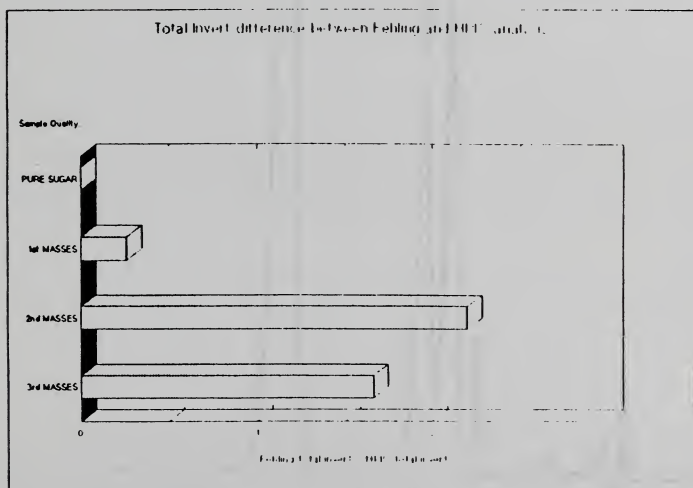
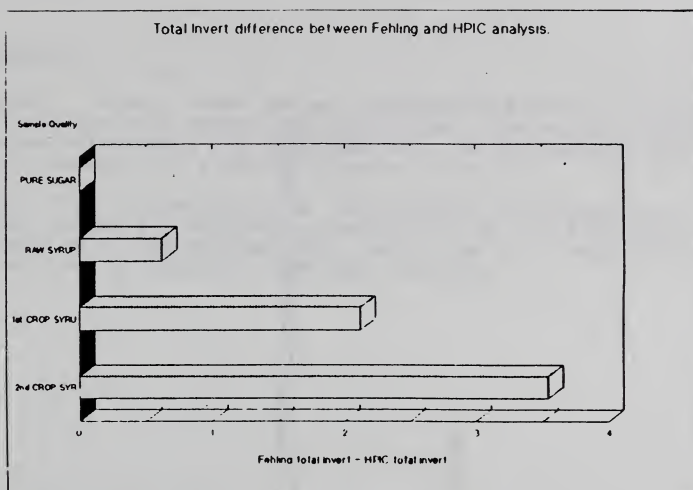


Figure 11 a and b. Comparison of Fehlings and HPIC results

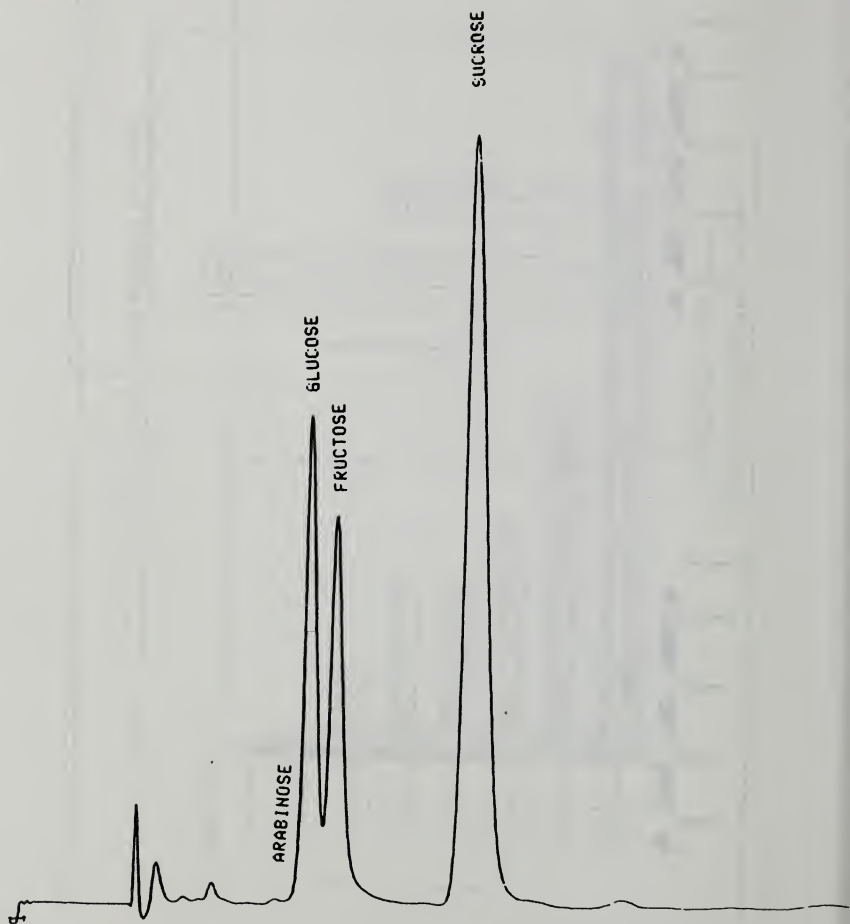


Figure 12. A typical molasses chromatogram by HPIC

DISCUSSION

Chairman: Since you are the only speaker here who has worked with both the AS-6 column the CarboPac PA1, let me ask you what pressure differences you have observed? Is the CarboPac more tightly packed with a smaller diameter material?

Plews: That is possibly true. The biggest difference we've noticed so far is the greater reproducibility and solubility of calibration of the CarboPac PA1. We are now at a stage where we may not have to calibrate with each analysis.

APPLICATIONS OF ION CHROMATOGRAPHY TO SUGARBEET PROCESS PROBLEMS

D. Eugene Rearick and Mary Little

The Amalgamated Sugar Company

INTRODUCTION

In our laboratory ion chromatography has been applied to a variety of sugar factory process problems and questions related to composition of factory process streams. Several examples of such applications including both single column and suppressed modes of ion chromatography are given.

GENERAL METHODS

All single column ion chromatographic determinations were performed using a Waters M-6000 HPLC pumping system, Wescan Anion/R column, and a Waters Model 430 conductivity detector. Other conditions were as described for each application.

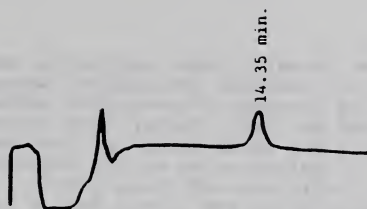
Suppressed mode ion chromatographic results were obtained on a Dionex Series 4000 system with Anion Micro Membrane Suppressor, using the column and eluent described, and either a Dionex Pulsed Amperometric Detector II or Waters Model 430 conductivity detector.

RESULTS AND DISCUSSION

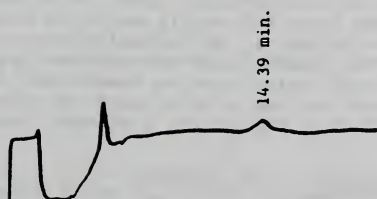
A. Anions in White Sugar

Although inorganic and organic ions are present in white sugar at very low levels ion chromatography is sensitive enough to give information on anions present (Tungland 1987). Several applications in our laboratory have made use of similar techniques.

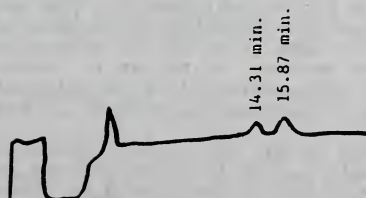
A sample of sugar showing unusually high turbidity was analyzed in parallel with normal samples using a single column ion chromatographic system. Samples were dissolved at 30-35% solids levels and 100 μ l was injected on a Wescan Anion/R column with 5 mM potassium p-hydroxybenzoate (pH 8.5) as eluent. Figure 1 shows chromatograms of three sugar samples: a low-turbidity sample from factory A; a low-turbidity sample from factory B; and a high-turbidity sample from factory B. All chromatograms show a peak at about 14.2 minutes attributed to sulfate at levels of 6-20 ppm in original sugar. The high-turbidity sample shows an additional peak at 15.87 minutes which matches oxalate anion in retention time. The original high-turbidity sugar, in this case, was calculated to contain oxalate at a 9 ppm level and it seems likely that turbidity was due to calcium oxalate.



FACTORY A
Low Turbidity



FACTORY B
Low Turbidity



FACTORY B

Figure 1. Analysis of high turbidity sugar

In a second application to white sugar analysis a sample of sugar unusually high in ash was analyzed for anions. Since excessive ash can be caused by the presence of potassium imidodisulfonate (Carruthers et al., 1957 and 1958; Parker 1960; Schoenrock and Johnson 1960), attention was first turned to that material. Imidodisulfonate was determined using an ion chromatographic technique reported by Littlejohn and Chang (1986). The strongly retained imidodisulfonate anion was eluted from a Dionex AG4A guard column only, with 6 mM sodium carbonate. In this case imidodisulfonate was found in high-ash sugar at only very low levels (less than 1% of the total measured anion equivalents). Anion chromatography on a Dionex AS4A column (elution with 18 mM sodium tetraborate) showed an anion profile of 6-8 ppm each of chloride, nitrate, and sulfate with smaller amounts (<1ppm) of malate, nitrite, and oxalate (Figure 2). A sample from another factory without ash problems showed a lower total anion level but little difference in relative levels of various anions. Comparisons of standard liquor samples from the two factories showed no major differences; in fact, standard liquor from the factory producing high ash sugar was actually lower in anions. In this case ion chromatography provided mostly negative evidence: that is, that a high concentration of imidodisulfonate or any other individual anion was not responsible for the ash problem. It was eventually concluded that sugar washing conditions resulting in an excessive syrup layer on the crystal surface were more responsible for the high ash than any single component concentration.

B. Scale Identification

Although anions in factory scale samples can frequently be identified by means of infrared spectroscopy (Rearick 1987), ion chromatography is a good alternate technique and has the advantage of providing simple quantitation of anions, especially in scales containing mixtures of compounds.

A typical evaporator scale sample was dissolved, with warming in 6N hydrochloric acid (0.1 g scale/50 ml acid), diluted ten-fold with water, and a small sample was eluted through a Dionex AS4A anion column with 16 mM sodium tetraborate. The chromatogram obtained (Figure 3) shows the expected large chloride peak at 1.8 minutes and peaks for nitrate (4.06 min.), phosphate (5.53 min.), sulfate (8.4 min.), and oxalate (10.84 min.). Oxalate is by far the major component of this scale (21.2% of original scale weight), with phosphate constituting about 5% of the material.

In the example described above, the major scale anion, oxalate, has a much higher retention time than the intense chloride peak resulting from the use of hydrochloric acid to dissolve the sample. If the chloride peak presents a problem, samples can be dissolved in hydrochloric acid and passed through a silver form cation exchange clean-up cartridge (Maxi-Clean IC-Ag; Alltech

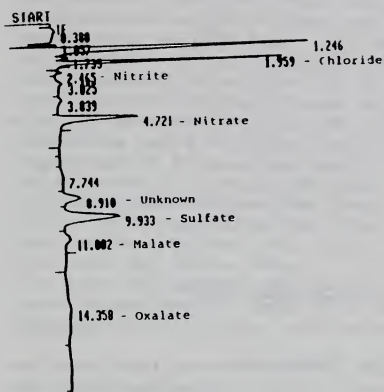


Figure 2. Chromatogram of high-ash sugar

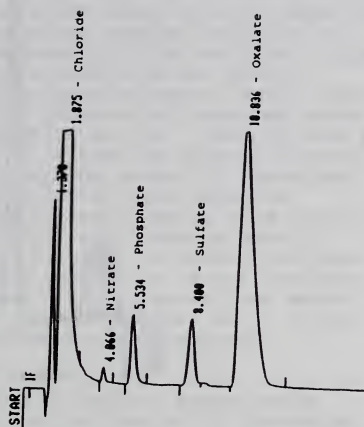


Figure 3. Chromatogram of scale sample

Associates, Inc.). This approach was applied to a second evaporator scale sample which was found to contain sulfate as the major detectable anion under the same chromatographic conditions as the previous example.

Another application of this clean-up technique was used to examine a precipitate from a sample thought by a customer to be beet molasses. Figure 4 shows chromatograms of a hydrochloric acid solution of the precipitate with and without clean-up. Treatment of the solution with a silver form clean-up cartridge removed essentially all of the chloride leaving only the major anion peak at 4.9 minutes. Based on the 4.9 minute peak, which corresponds to a very high phosphate concentration, and other analytical data it was concluded that the material was not beet molasses.

In using such a cleanup technique it should be kept in mind that silver salts of some scale components may also be insoluble and could be at least partially removed during cleanup. This possibility has not been fully investigated in our laboratory. In general, the common scale-forming anions found in beet sugar factories are easily separated from chloride without sample cleanup.

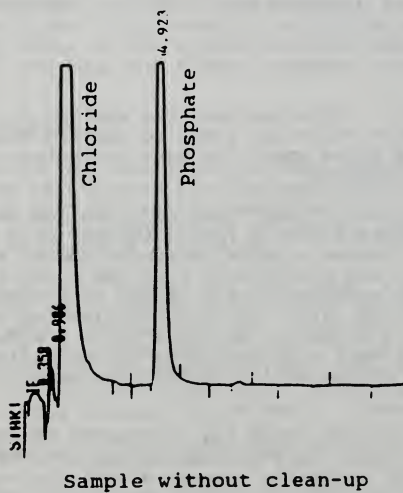
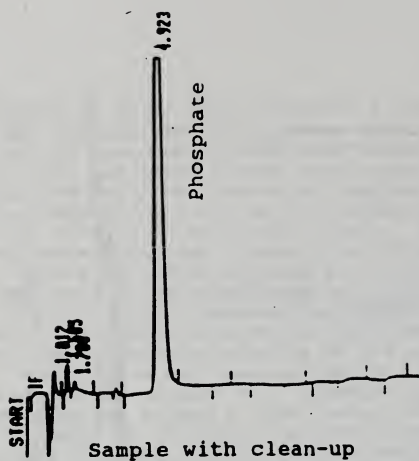


Figure 4. Analysis of unknown precipitate

C. Amino Acid Determination

The Dionex Pulsed Amperometric Detector is widely applied for carbohydrate determination but is not specific for carbohydrates. The detector responds also to amino acids which in some cases may be separated using columns and conditions similar to those for carbohydrates (Rocklin). In our laboratory this was first viewed as a problem since during attempts to determine low levels ($<0.2\%$ /dissolved solids) of invert in beet molasses, amino acids were a serious interference with the glucose and fructose peaks eluting from the Dionex AS6 carbohydrate column. Recently due to questions about sugarbeet quality as related to various factory processes it became desirable to determine levels of individual amino acids in process samples, principally beet molasses. Although good methods for ion chromatographic amino acid determination have been reported (Small, 1989) such methods usually involve post column reactions with ninhydrin or another reagent specific for amino acids. Since our laboratory is not equipped for such procedures the decision was made to investigate amino acid determination using available equipment; that is, a pulsed amperometric detector and carbohydrate analysis columns.

As mentioned above the Dionex AS6 carbohydrate column, which is an anion exchanger, gives separation of several amino acids. Best results were obtained with a gradient employing increasing sodium acetate concentration in a 0.2 N sodium hydroxide eluent. Pulsed amperometric detector parameters (applied potentials and pulse durations) were adjusted to maximize detector output for amino acids. Figure 5 shows a typical chromatogram of a beet molasses sample with several amino acid peaks identified. This system proved to be most effective for the highly retained amino acids, glutamic acid, aspartic acid, and tyrosine, which are all of major importance in beet molasses. More weakly retained amino acids are subjected to interference from carbohydrate peaks.

Amino acids can also be separated on cation exchange columns and in our case reasonably good results were obtained with a conventional calcium-form cation exchange column normally used for carbohydrate determination (HPX-87C, Bio-Rad Laboratories). Amino acids are eluted from an HPX-87C column with low ionic strength solutions or even water. However, since a high pH (pH 11-13) is necessary for pulsed amperometric detection, a base solution (0.3N sodium hydroxide) was added post column. This addition of base requires no special equipment other than a pump and mixing coil normally supplied as part of the Dionex pulsed amperometric detector system. (This use of the amperometric detector with a column other than an anion exchanger, such as the Dionex AS6 column, is unusual and could also be applied as a carbohydrate detection method to allow use of the highly sensitive amperometric detector with practically any column system.) Using this system on beet molasses gives a good separation of several of the amino acids that cannot be determined on the AS6

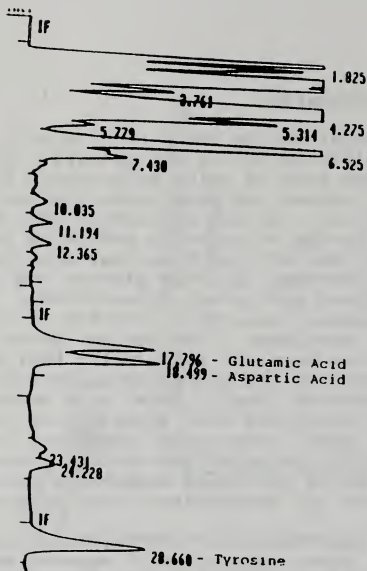
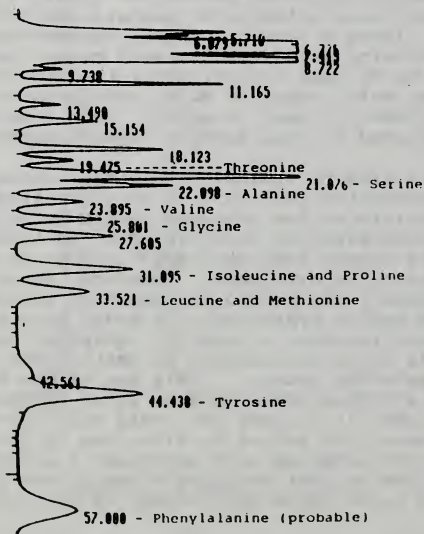


Figure 5. Chromatogram of beet molasses (anion exchanger)



STOP

Figure 6. Chromatogram of beet molasses (cation exchanger)

column, as shown in Figure 6. Under the conditions used for the chromatogram (eluent: water; column temperature: 82°C) glutamic and aspartic acids elute early, under the carbohydrate peaks. Thus for complete determination of beet molasses amino acids, two column systems must be used. Our work on amino acid analysis methods is still in progress and hopefully further refinements of the HPX-87C column method will be made.

D. Investigation of Carbohydrate Polymers

During the 1989-90 campaign a recurring problem with a flocculent precipitate in a factory process system was thought to be related to microbiological growth since it seemed to occur at low process temperatures. However, the material did not have the gelatinous appearance of dextran but under the microscope had the appearance of a mass of short fibers. The infrared spectrum of the material indicated it to be a carbohydrate but did not match any dextran sample available. In fact, the infrared spectrum resembled that of dried sugarbeet pulp except it lacked the carbonyl band of pectin. Although the material did not contain pectin the possibility remained that it was a sugarbeet hemicellulose.

To gain further information about the structure of the polymeric carbohydrate it was decided to hydrolyse the material to simple carbohydrates and use ion chromatography to compare the hydrolyzate composition with those from known carbohydrate polymers. For comparison a commercial dextran sample was hydrolyzed by refluxing four hours in 0.5 M sulfuric acid according to a published method (McAllan, 1985). Ion chromatography of the hydrolysis product (Dionex AS6 carbohydrate column, 0.02 N sodium hydroxide eluent, pulsed amperometric detector) showed only glucose, at a recovery of 95%. A similar hydrolysis of a commercially available hemicellulose, arabinogalactan (Sigma Chemical Company, from larch wood), gave an approximately 1:5 ratio of arabinose to galactose. Hydrolysis of a dried sugarbeet pulp sample was also carried out and a chromatogram of the hydrolyzate is shown in Figure 7. From the 75% of the original material which dissolved the principal monosaccharide obtained was arabinose along with smaller amounts of galactose, glucose, and xylose. This agrees with data reviewed by McCready (1966) which indicates high levels of araban in sugarbeet pulp. Finally, hydrolysis of the carbohydrate precipitate from our process system gave, as shown in Figure 8, an approximately 2:1 ratio of glucose to xylose with a trace of arabinose. Only about 30% of the material dissolved under the hydrolysis conditions.

Based on hydrolysis behavior and the fact that the hydrolysate contained a significant level of xylose it was concluded that the precipitate was definitely not dextran. The composition seemed to show that the material was a sugarbeet hemicellulose precipitated under certain process conditions; however, the conditions

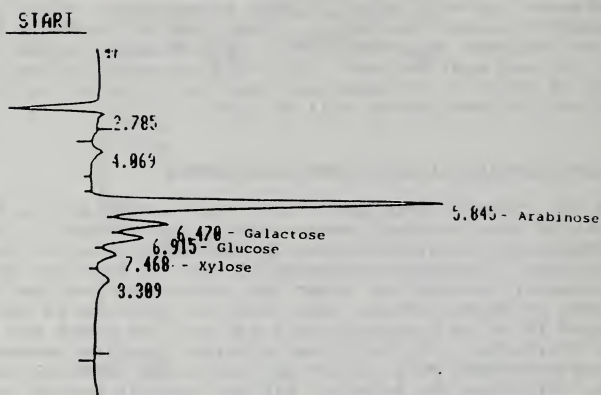


Figure 7. Chromatogram of sugarbeet pulp hydrolyzate

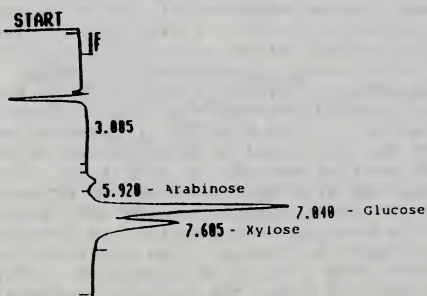


Figure 8. Chromatogram of precipitate hydrolyzate

of precipitate formation in the factory still indicated microbiological growth. The possibilities remain that the material was either an unusual xylose-containing product of microbiological growth or a co-precipitate of a microbiological product and a hemicellulose. Polysaccharides produced by bacterial growth and containing saccharides other than glucose have been reported (Kitchen et al., 1986). In spite of the fact that the origin of the precipitate we examined was not definitely established the technique of polysaccharide hydrolysis and carbohydrate identification by ion chromatography was used to provide valuable information on the composition of an unknown polysaccharide process sample.

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DISCUSSION

Question: At what place in the process did you find the polysaccharide you mentioned?

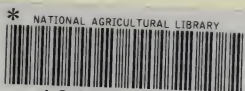
Rearick: It was found in the juice softener system in one of our factories. We thought it might be a hemicellulose because there is a pH change in the process at the point where this material was precipitating out. However, it appeared to occur only at low temperatures so we think it is probably a microbiological product.

Question: On the hydrolysis rate that you obtained with sulfuric acid: you quoted 30%. Does this mean that you could account for only 30% of the product? If there are uronic acid groups in the polysaccharide, they are chewed up by the sulfuric acid and don't show up as products.

Rearick: Only 30% went into solution - we did not try to quantify all the products.

Question: On the chromatogram of inorganic anions, there was an unknown peak - we've observed this too, and find it to be carbonic and/or lactic acid - organic peaks showing up in an inorganic analysis. This happens also with HPLC, but is a greater problem in IC, because if the unknown substance has a retention time similar to an inorganic ion in the determination, it can interfere. We can use regular HPLC to check the identity of these peaks.

Rearick: Yes, in fact we've found a malate peak in that chromatogram. We've seen oxalate also.



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